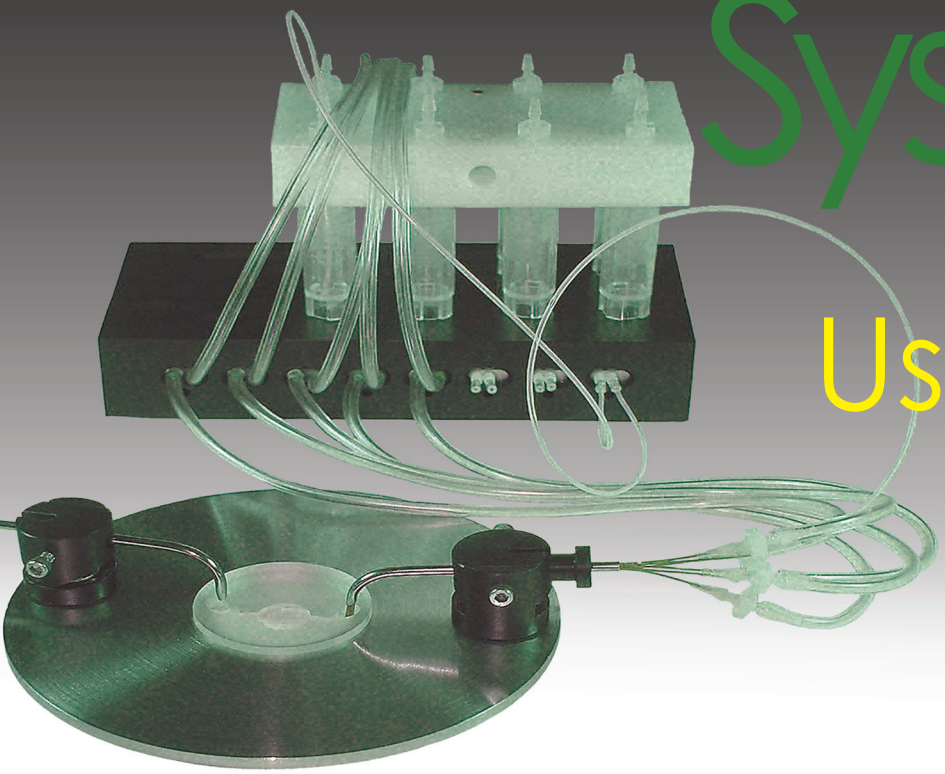
Perfusion Systems



User's Guide

Programmable Systems for Solutions Application & Switching

- **Up to 16-Channels**
- **Programmable timing and sequencing up to 32 steps**
- **Compatible with Imaging & Data Acquisition systems**
- **No electrical noise during switching**
- **Automatic switch to WASH solutions for easy manual operation**
- **Bath Perfusion & Local Application**
- **Works with Temperature Controlled Systems**

Bioscience Tools

Ph: 877-853-9755
www.biosciencetools.com

Contents

Specifications

Output
x2 DB-9 connectors for 16 solenoids, up to 35V (12V standard), 5A max current per channel

Remote Control
wireless control for easy manual operation

TTL Signals
+5 Volts activates valves, individual BNC connector for each valve, DB-9 connector for the first 8-valves

Analog Voltage Input Decoder
Input analog voltage at rear panel BNC is decoded at 0.5 V intervals per channel

Pressure Controller
0-15PSI (PC-16P model only)

Programmable Timers
1 ms accuracy, stored in controller memory

AUTO memory mode
automatically switches channels, and activates “wash” solution between channels in sequence

Software control
through RS232 port (or USB adapter)

Port Diameter
Luer connectors (pinch and solenoid valves)
barbs for 1/16in I.D. tubing (pressure switch)

Valves
12VDC solenoid;
12VDC pressure switches;

Size (Valves)
Solenoid Valves 12L x 2.5W x 2.4H in. aluminum box
Pinch Valves 12L x 2.5W x 2.4H in. aluminum box
Pressure switches 5L x 2W x 2.4H in. aluminum box
Includes X-blocks to mount on 0.5” O.D. stands

Size (Controller) : 12Wx6Hx9D in.

Power Supply
100 to 240 V AC, 50/60 Hz
12VDC output (up to 35V output can be configured for custom valves)

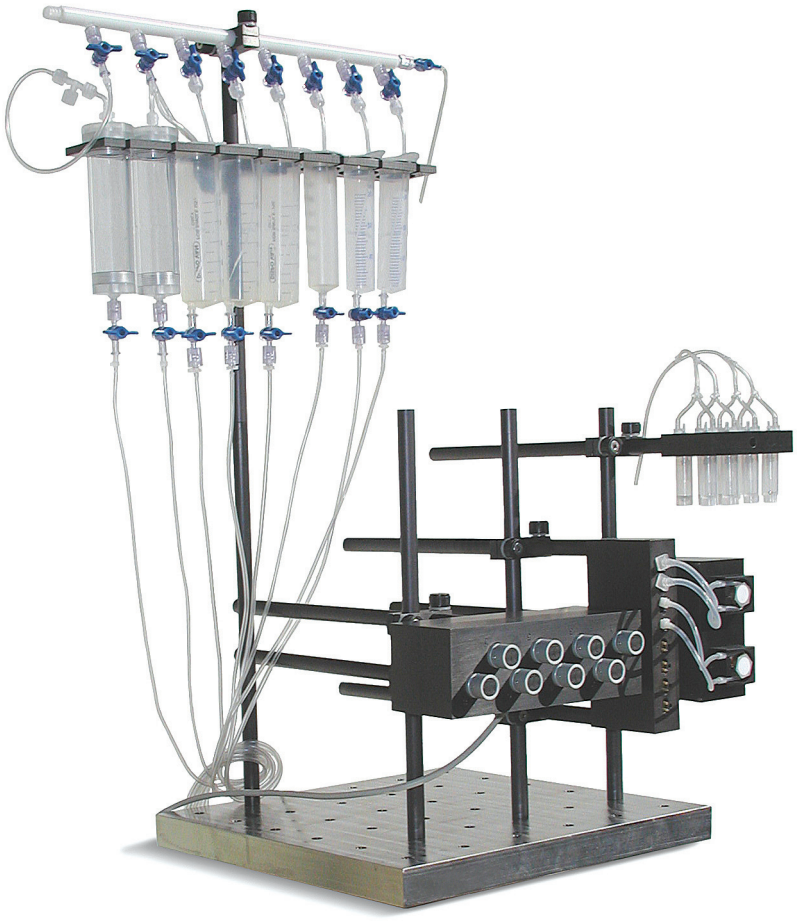
CE

Introduction

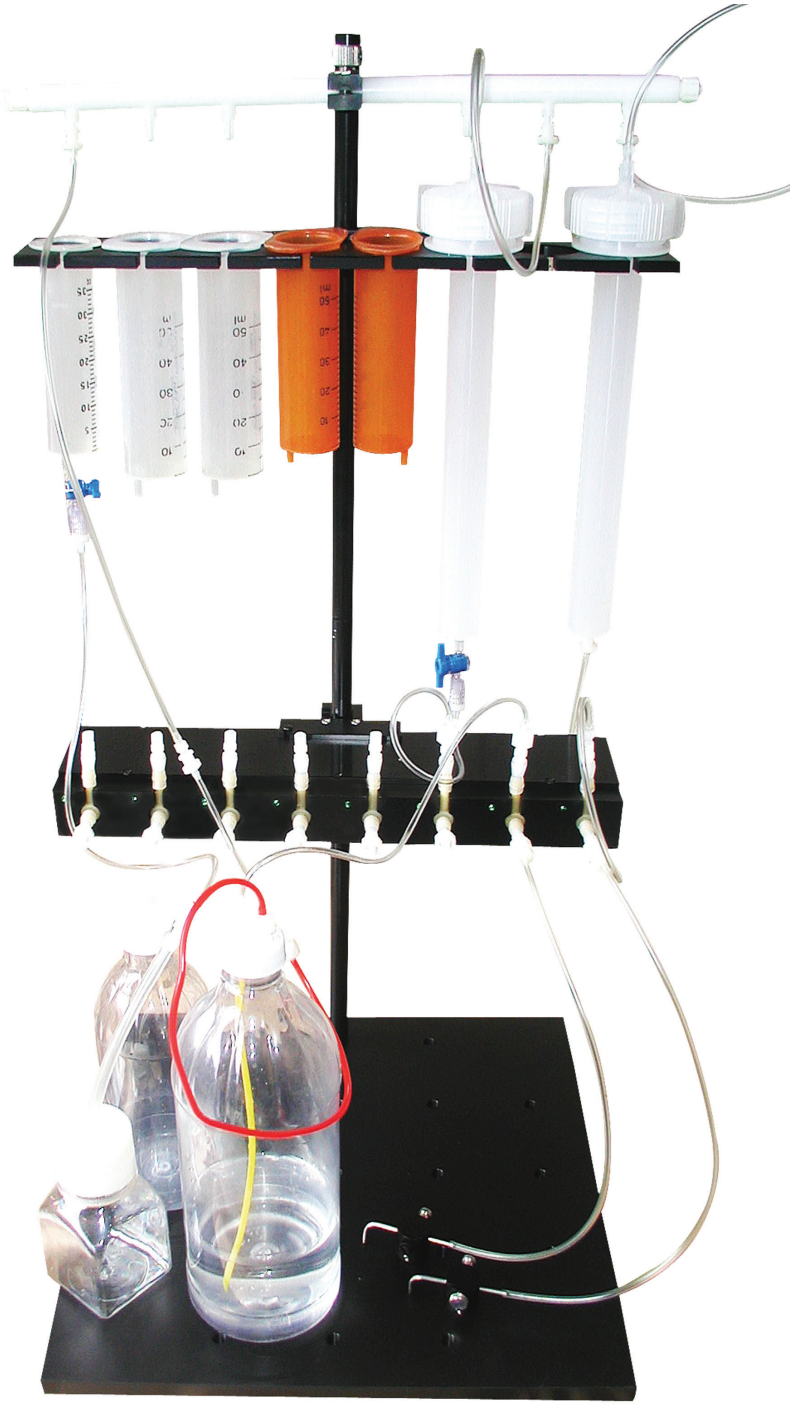
The complete perfusion system comes with a 16-channel controller, a power supply, valves mounted in aluminum box with connecting cable, mounting rods and/or X-blocks, a syringe holder, syringes, stopcocks, tubing, a manifold, and all necessary fitting. The small size allows you to position the valves near your sample using included magnetic syringe holder and provided X-blocks. Alternatively, the valves can be mounted on an anti-vibration stand of included SH-1A syringe holder, in case you do not have magnetic surfaces. The detailed instructions on syringe holder, and optional gas mixture delivery adapter and pressurized cylinders assembly are in appendixes at the end of this book. The following are an illustrated installation guide for the perfusion system and example configurations of experimental perfusion setups.

Installation Guide

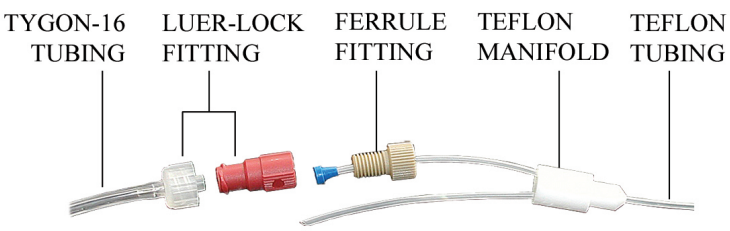
1 Using provided mounting rods and X-blocks, mount syringes for solutions and valves on vibration isolation board of SH-1A holder, or on included magnetic stand. Shown on the picture below is SH-1A board with syringe holder, gas delivery adapter, small volume delivery system SVDS1, pinch valves, solenoid valves, and 2-channel flow control unit.

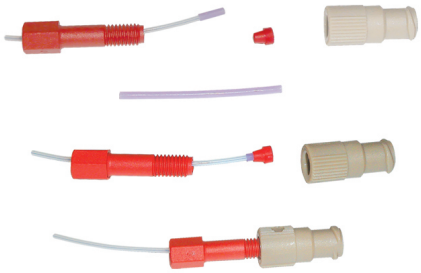


2 Before connecting tubing to the valves, mount the valve box on vertical rods attached to the board or the magnetic stand included with SH-1A syringe holder. Refer to Appendix for instructions on how to connect tubing to the syringes.



3 To connect miniature manifolds use luer-adapters, or adapters from optional fitting kit PS-KIT. Tygon tubing can be connected to barbed luer-fitting included with SH-1A syringe holder. To connect to TPM manifold, use ferrule based luer-lock barbed fitting to splice to polyethylene tubing, which fits over Teflon tubing inserted inside TPM manifold.

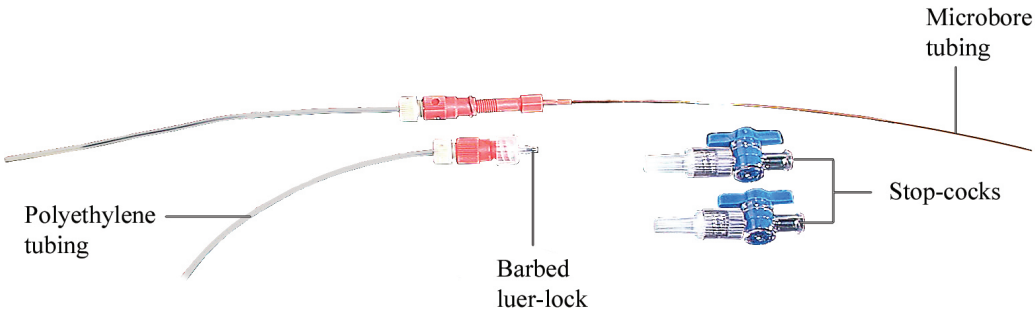




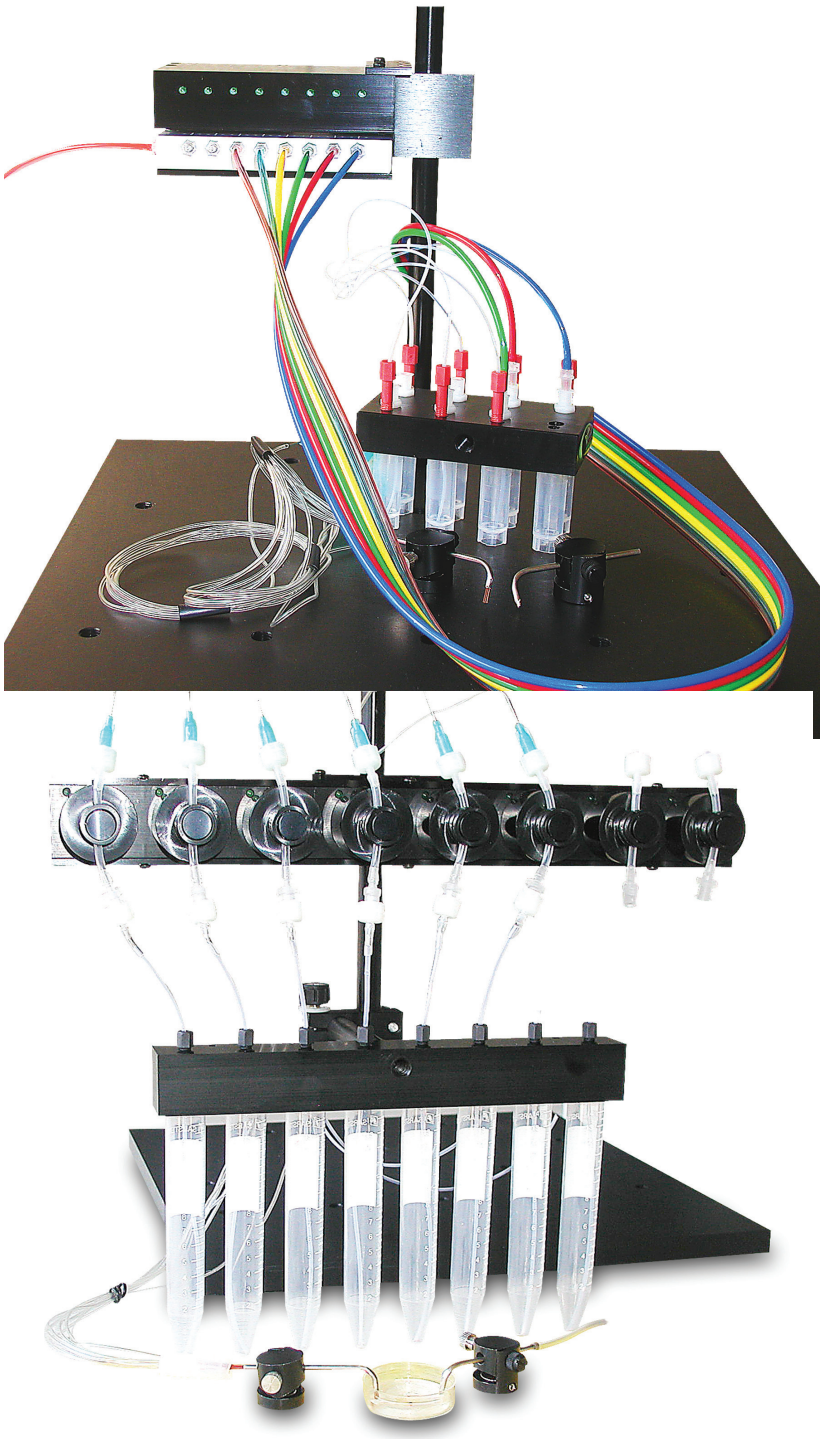
Perfusion Systems can be connected to thinner microbore tubing using optional fitting, easy-disconnect luer-locks and ferrule fittings.

NOTE: Polyethylene tubing connects over teflon tubing of perfusion manifolds; the other end of tubing connects to the valves of the Perfusion System.

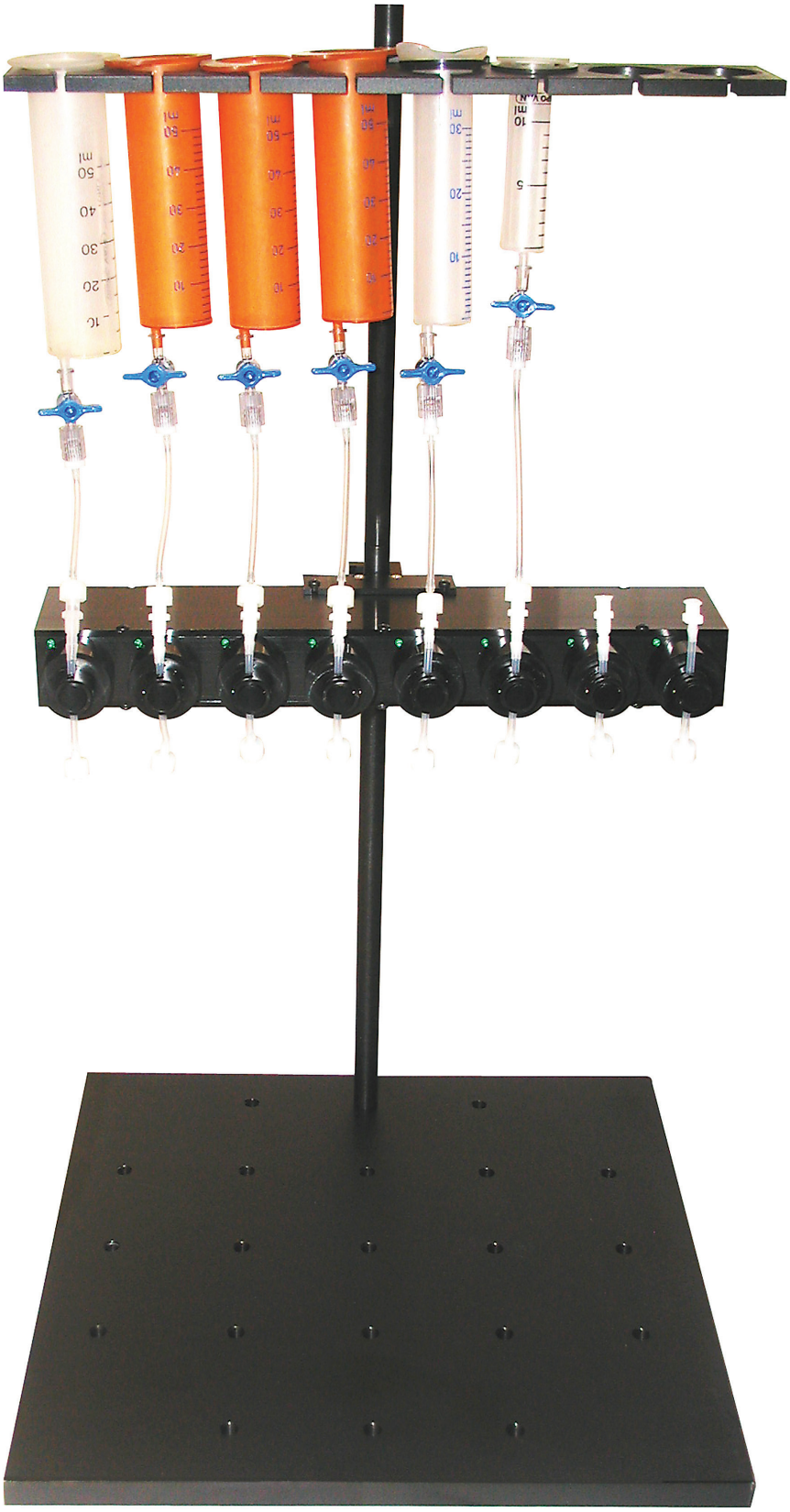
NOTE: Tygon 1/16" I.D. Tubing can also be connected to manifold and polyethylene tubing using provided ferrule and luer-lock fitting..



4 After connecting all tubing, your system should look similar to the pictures below: the configurations include pressure switch PS-V8P (top) or pinch valves (bottom), which can be programmed deliver pressure to eight different solutions in SVDS1 manifold (small volume delivery setup - top) or SVDS2 (bottom). The switch requires a pressure input (on the left) from built-in pressure controller/pump (PC-16P model). Tubing from SVDS1 manifold deliver solutions through zero-dead volume manifold ZMM to your sample. A suction tubing of MTH-S magnetic holder is used for outflow of solution from the sample chamber. On the bottom - all solutions are pressurized and delivery happens when pinch valves open.



PS-8H Pinch Valve System should look similar to this:



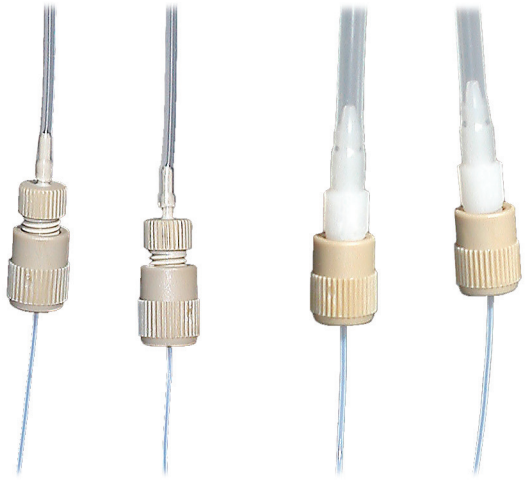
5 Small Volume Delivery system SVDS1/2 can be also mounted on vertical rods using the provided mounting rod and X-block. See Appendix SVDS1.

6 The valve cable should be connected to OUT 1 on the back of the controller (or OUT 2 for more than 8-channel systems).

Connect the power cord to the controller and plug the cord into 120/220V outlet. Turn the controller ON using ON/OFF switch on the BACK panel. Initially the controller will keep valves closed, which is shown by light green OPEN button. Touching the button OPEN, or clicking on any channel on provided remote control, will open the valves - the button will show CLOSE word.

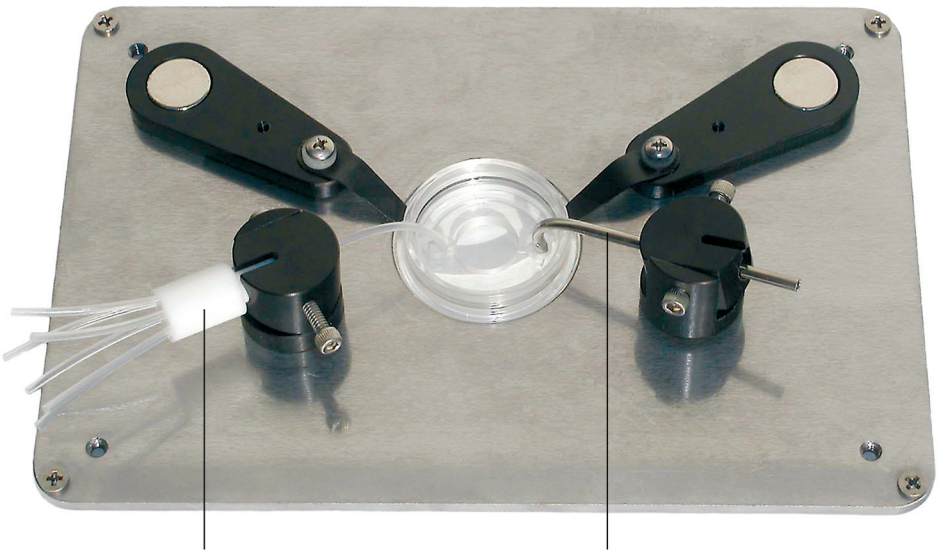
Fill the reservoirs/syringes with solutions. Start operating the system by opening the valves manually by touching the appropriate buttons on the front of the controller (or by using the remote control). This should fill the lines with solutions. Note: an optional source of pressure PC-R15/10 might be required to push solutions through the lines (for SVDS1/2 systems, for example).

7 Before using the perfusion system with open chambers, you need to configure an outflow lines. Our small controlled flow units CFPS-1U66 (below, left) can be mounted on the same vertical rods, using provided X-block. Insert provided soft tubing inside the unit while it is rotating slowly. Connect the ends of soft tubing to Tygon or polyethylene tubing of the perfusion system using provided luer, barb fitting or ferrule based adapters, or optional fitting CFPS-KIT shown below (on the right).



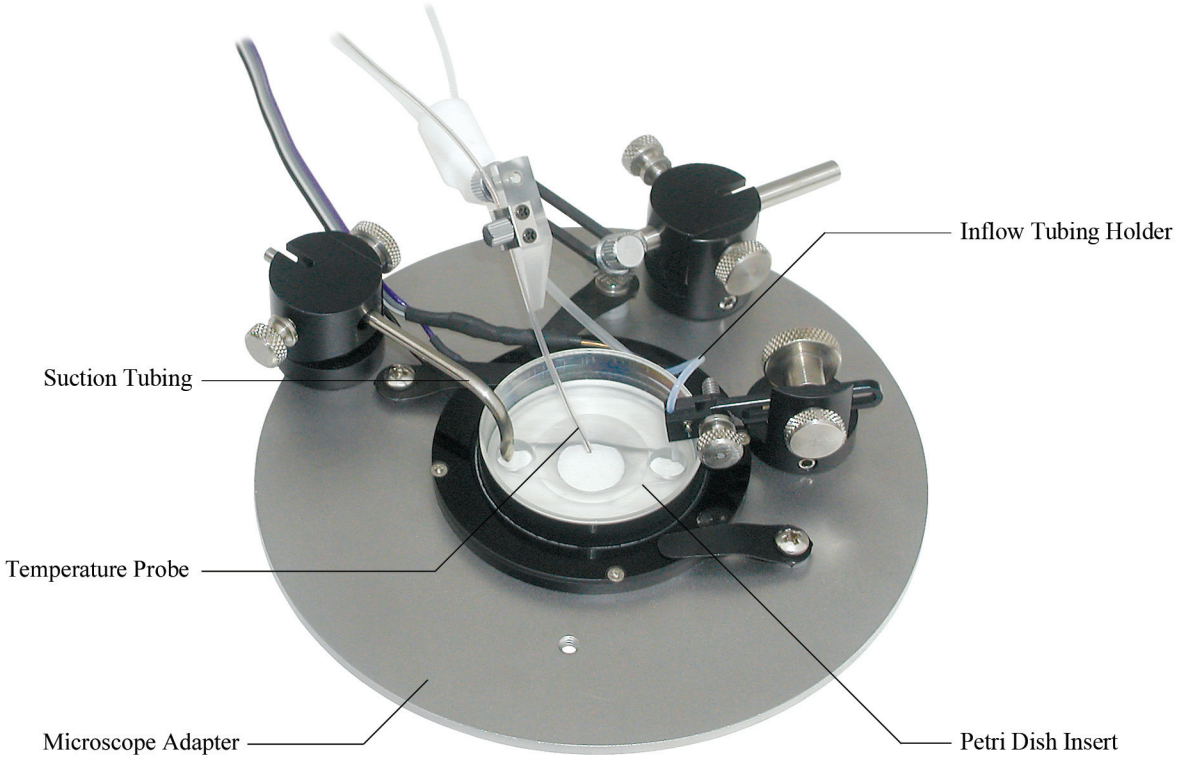
The following are examples of connecting the perfusion system to your sample placed in petri dishes or on coverslips. Inflow tubing can go through the heating element and then fixed near the sample using a miniature holder. Optional stainless suction tubing on magnetic holder MTH-S provides smooth solution outflow. In case when even a slight contamination of different solutions is undesirable, a zero-dead volume manifold can be used instead of regular manifolds. The zero-dead volume manifolds offer an additional advantage of facilitating perfusion and accelerating solution exchange rate around your samples (see Appendix ZMM). This manifold can be also used for solution outflow/suction, by connecting one of the channels to CFPS-1U66 unit.

8 The included manifold TPM (see Appendix TPM) can be mounted next to your sample using an optional magnetic holders MTH attached to a microscope adapter MA. The manifold can be also connected directly to the inflow tubing of heating stages.

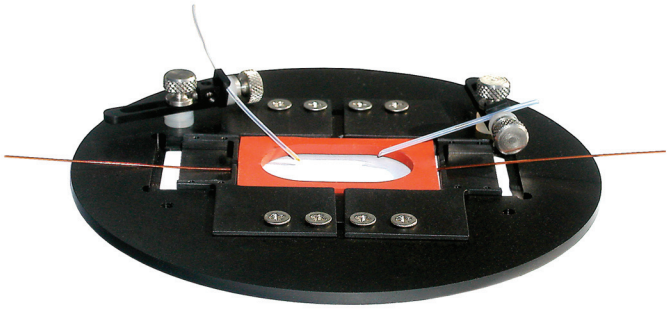


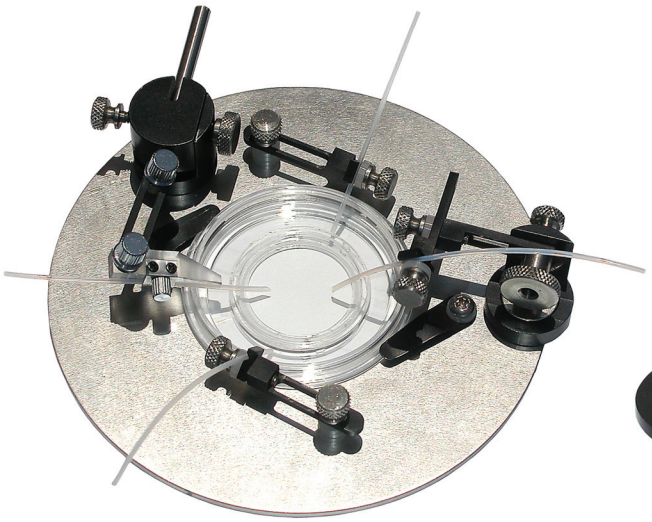
Perfusion Manifold (TPM) Mounted inside Magnetic Holder (MTH)

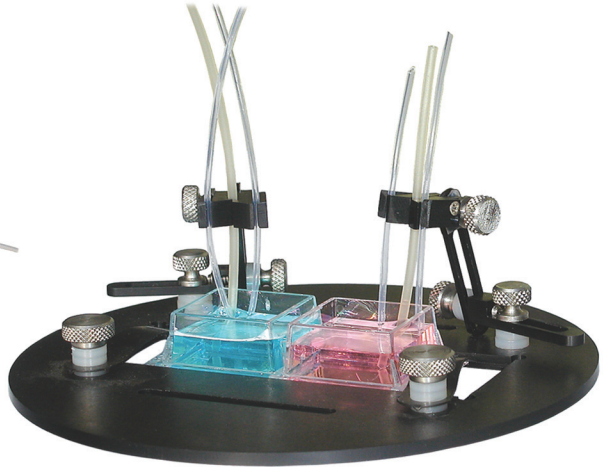
Suction Tubing mounted inside another Magnetic Holder MTH-S



9 Miniature magnetic and screw type holders provide convenient options to arrange inflow and outflow tubing around your sample. An extremely flexible multi-tubing holder MH-2 can fix several tubing firmly in required positions. Miniature sizes of the holders allow you to fix inflow and outflow tubing around complex chambers, including chambered coverglasses. Below are additional examples of arranging perfusion tubing around your samples. On the bottom is double perfusion of membrane support inside a glass bottom dish.







Valve Control

Each output channel can be activated by one of four ways:

Manual Switch positioned on the front display, or using remote control; manual control can be used to OVERRIDE external input;

External Input TTL signal activates an individual channel. Rear panel BNC connectors provide access to all 16 channels. Rear panel DB-9 connectors provides a convenient access through an optional DB-9 cable to activate the channel using digital signals (parallel to the BNC connectors);

Analog Signal Decoder allows you to activate each output channel by applying a voltage signal in the range 0 - 10 V to Analog Discriminator input BNC, located on the rear panel; using this input, one analog output channel from a data acquisition system can control 16 output channels;

Binary Decoder allows using four digital output channels from a data acquisition system to control 15 output channels; the 16th channel is activated when no input signal is present.

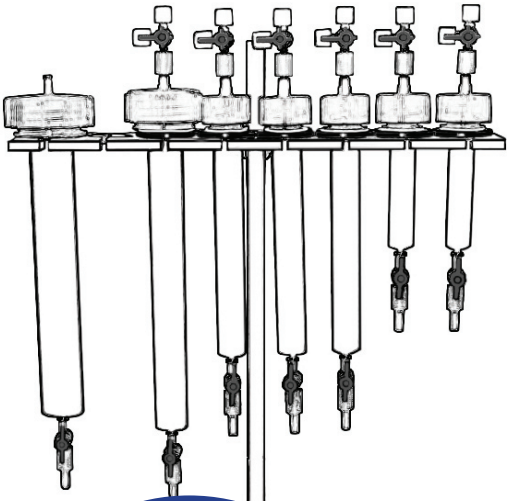
Software Commands sent through RS232/USB interface allow you to program solution application and exchange protocols, which make the system extremely flexible.

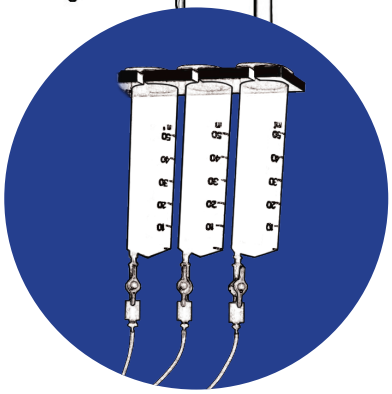
Valves

The solenoid and pinch valves come with luer fittings installed. These allow customers to connect to any tubing using luer connectors, which are included with complete systems (or in PS-FIT kit). Pressure switch comes with barbed fittings installed to connect to 1/16in I.D. soft tubing.

The pinch valves will open only if the “pinch” tubing is installed inside. In order to insert tubing inside the valve, activate the valve first, which will be shown by GREEN indicator next to the valve, and gently push the piston inside the valve, using a flat screw driver, for example. Insert tubing while the valve is open.

Note: If you experience undesirable hydraulic disturbance during valve switching, which might destroy your sample, you can alleviate these fluctuation by putting an air bumper just after the valve’s outlet. A simple bumper can be constructed using a T-connector with the middle outlet plugged. The other two outlets should be connected inline with the solution flow. After filling the T-connector with solution, an air pocket will form inside the plugged outlet, which will dampen the pressure fluctuations.



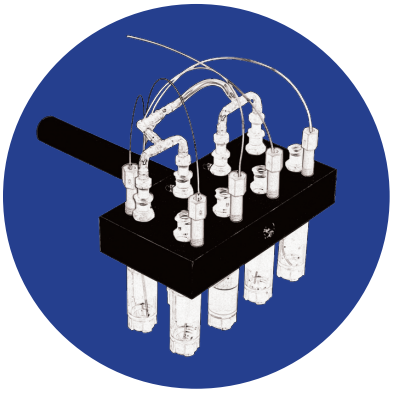


Complete Functional Perfusion Setup

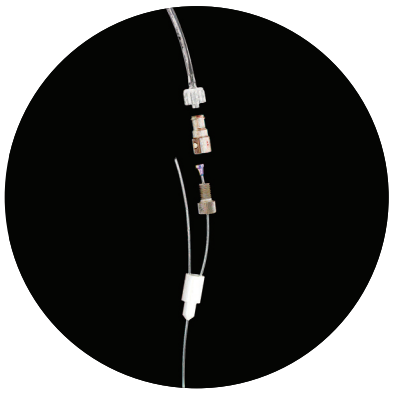
includes the following components:

1. Reservoirs with solutions and solution holder, the purpose of which is to elevate solutions above your sample if gravity is used to deliver the solutions. The solutions can be also pressurized using pressure cylinders PC, on the left, which eliminate the need of elevating solutions. The solutions can be also saturated with gas mixtures using adapter SH-A, shown on top of the center picture.

A common option is a traditional syringe holder SH-1A, which requires a magnetic surface. Alternatively, the system can be mounted on a stand of SH-1A, shown in the center picture.

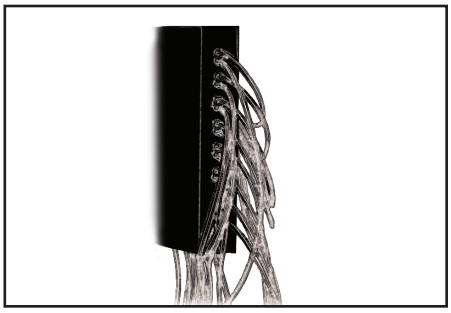


In order to deliver small volumes of solutions available in limited amount use SVDS1/2 system, which require a source of pressure PC-R15/10 to push solutions or Controlled Flow system CFPS to withdraw solutions from disposable tubes.

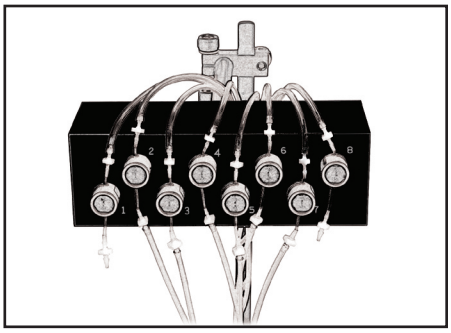


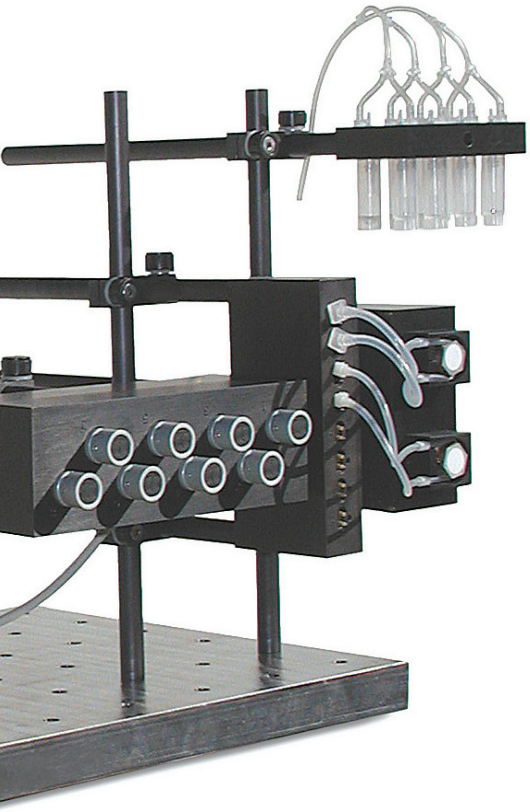
2. Each system includes a set of tubing, manifolds and fitting accessories to provide easy connections and solution flow to your sample.

3. Solution switching is accomplished using solenoid valves. Use inexpensive solenoid valves or pinch valves. You can also use pressure switch, which delivers pressure to solution to drive liquids to your fluidics devices. The valves connect to the 16-channel controller, which allows you to switch solutions manually or using your data acquisition system and software.

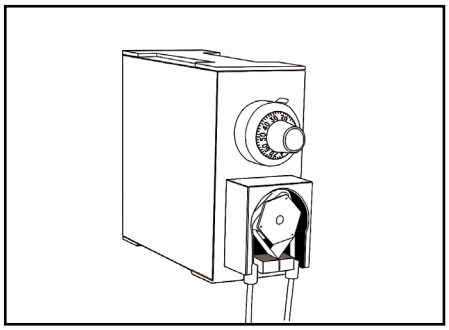


In the pinch valve, a soft tubing is squeezed closed by the valve, and opens when the channel is ON. The results are simple tubing replacement and easy system cleanup after experiments. Recommended for strong solvents and reagents, and for hard to wash/clean solutions.





4. To complete the system you need to configure solution outflow, which is required in the open systems - petri dish, for example. The controlled flow unit CFPS-1U66 can handle up to 22ml/min. Outflow starts automatically, every time perfusion is ON, to prevent accidental solution overflow in the system. Multiple units can be used to control inflow rate as well. Shown in the middle picture is a 2-channel unit from a computerized CFPS-2 system, which allows you to automate solution flow using data acquisition systems. Computerized packages with software to program any solution exchange protocols are also available. Note: closed fluidics devices might not require outflow, as solution can be pushed through using pressure from PC-R15/10 pumps.



Pressurizing Solutions

If your experiment requires applying pressure to solutions, you can use either a small volume delivery system SVDS1/2 system, or pressure cylinders PC-10/50. SH-A pressure manifold can be used to connect pressure cylinders PC-10/50 to a pressure source PC-R15/10. Pressure is applied to accelerate solution flow through microfluidics devices, or to push solutions when no gravity is used. NOTE: the pressure source is built inside PC-16P controller.

Maintenance

The solenoid valves need to be washed with distilled water to prevent precipitation and particles deposits after the experiments. Simply replace the test solutions with distilled water, and let all water flow through your system. This not only will clean the valves, but the whole perfusion system as well. If you did not do this after each experiment, you might also have some infection with yeast, for example.

The valves can be cleaned using a regular syringe connected to a piece of tubing to fit the valve's barb. While valve is open, push the content out of the syringe to generate a strong stream of the water through the valve. Repeat, by reconnecting to the other barb. Repeat for each valve.

If you have infection inside the valve, you might do an extra cleaning with a detergent solution. Let the valve soak in detergent for some time before cleaning with distilled water.

If you washed the system regularly, you should not have any contamination (otherwise any lab detergent should help). Alcohol does not dissolve the tissue but preserves it. If you use regular buffer with well soluble salts, the valves should be fine after DISTILLED water wash out.

The problem might arise when there are some particles in the solution. When the valves are not washed, for examples, the salt might crystallize. If you use colloid solutions, the particles might concentrate inside the valve. Then you might need a special solvent to dissolve the particles. Use pinch-valves system with hard to wash solutions for easy maintenance.

Sometimes, bubbles can be trapped inside the valve. You should remove all bubbles from tubing (by slightly hitting the tubing with fingers), because this is a common problem resulting to reduced solution flow. A common way to remove bubbles is to preheat solutions after taking them from a refrigerator.

Using Wireless Remote Control PC-16-RC

1. The remote control unit requires three AAA type batteries (included).

2. Eight push-buttons will activate/de-activate the first eight channels of PC-16 controller directly: push once to activate, push again to de-activate.

3. The grey unlabeled, CONTROL, button will activate/de-activate channels from 9 to 16, when used in conjunction with buttons 1 to 8. Pushing the control button will make the remote unit's LED turn red. Pushing the numerical button, while LED is red, will activate/de-activate the corresponding channel of PC-16 controller.

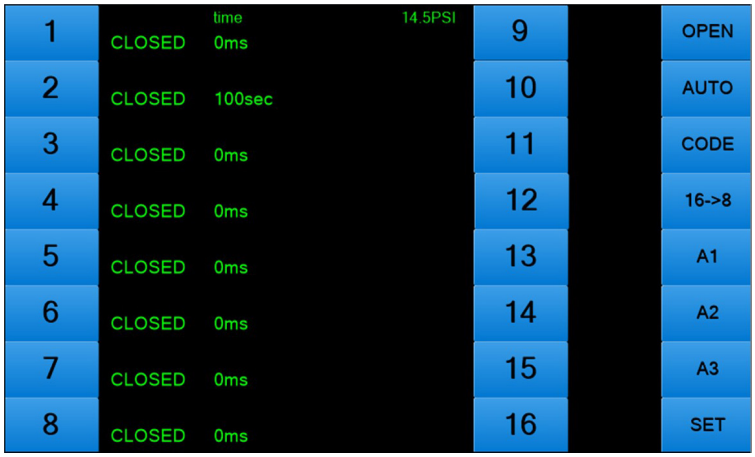
The remote control is programmed to activate only PC-16 controller with certain address (and will not work with other controllers). To put the remote into the correct mode, press GREY (CONTROL) button until the remote starts flashing (blinking) and then press button "1" - to program to the address 1 (or 2 for address 2; and 3 for address 3). To program the controller for the correct address, touch button A1 for address 1, or A2 for address 2, A3 for address 3.

	PC-16 CHANNEL	PC-16 CHANNEL
BUTTON		CONTROL
1	1	9
2	2	10
3	3	11
4	4	12
5	5	13
6	6	14
7	7	15
8	8	16

Troubleshooting

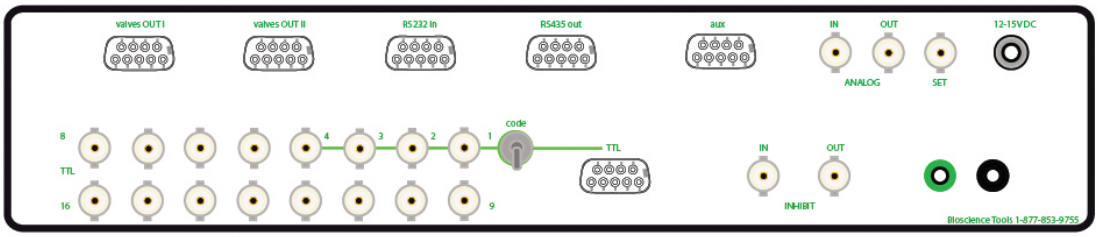
If the remote unit stops activating some channels, press the control button until the unit's LED starts blinking and push button 1 to restore the settings.

Front Panel Controls



Front Panel Controls	
1,2, ... 16 Switches	Touching the channel NUMBER button manually switches valves ON/OFF. This will override external software, TTL and ANALOG signals.
AUTO CLOSE CODE	Switches the controller into Automatic Memory Mode. Activating any channel manually (or using remote control) while in AUTO memory mode - will initiate previously set timer for this channel, and starts switching next channels in sequence if programmed. Pressing AUTO button while a channel is OPEN - will designate this channel as a "WASH" solution, This channel will turn ON automatically when no channels are open. This can be used to activate "WASH" between channels in AUTO sequence. Pressing CLOSE turns INHIBIT mode ON. In INHIBIT mode, there is no signal or power supplied to the valves - valves are CLOSED . This is used if turning all the valves OFF at once is required during the experiment, or during the initial setup and filling the lines. CODE is used to switch the controller into CODE mode;.
A1, 2, 3	Switches the address for the controller, this should correspond to the address set on the remote control;
SET	Displays SET screen to adjust timers and sequence parameter;
16 - 8 / 8 - 16	Changes the order of channel buttons on the display, this button is visible only if 16-channel configuration is activated on SET screen

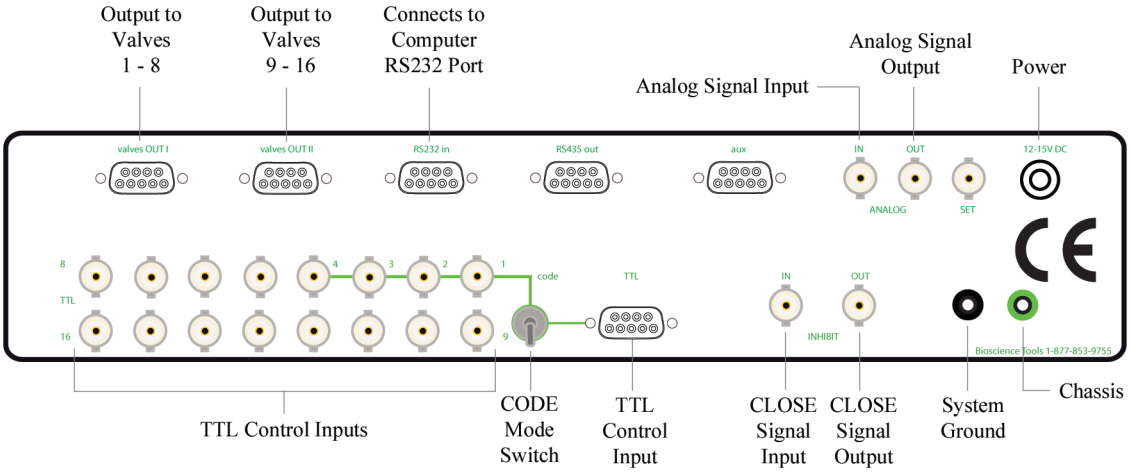
Output and Back Panel



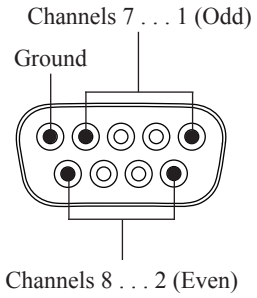
Outputs	
DB-9 Connectors VALVES I VALVES II	Two DB-9 connectors on the rear of the controller are used to connect to the valves. Valves I to valves 1-8, and Valves II to valves 9-16.

Back Panel	
Valves Out I, Out II	DB-9 connectors to valve's cable for 1-8 and 9-16 channels.
BNC 1 ... 16	BNC connectors for TTL input signal (+5V). Applying the TTL signal will activate an individual channel. Multiple parallel connections are possible.
Code	This toggle switch will turn the controller into Encoding Mode, which will allow you to use only 4 TTL inputs to switch 15 channels (the sixteenth is active when no signal is present). Manual switch on font will override this.
Analog IN SET	IN: BNC connector to apply an analog signal to switch individual channels using Analog Discriminator. SET provides 5V output when any valve is OPEN, and can be used to activate outflow unit CFPS-1U66.
Inhibit	IN: BNC connectors to apply an external signal (+5V) to CLOSE the valves (INHIBIT mode). OUT: BNC connector to provide output 5V signal. This can be used to link multiple devices together.
RS232 IN	DB-9 connector for the provided null-modem cable to link to your computer and use software control (during imaging applications, for example). Can be used with optional USB adapter.
Green Jack Ground Black Jack Box	This is Circuit Ground, which is connected to the analog and digital signal grounds, and to grounds of valve outputs. BLACK jack is connected to the outside of the controller: the box.

Connector Specifications



This is the diagram for OUTPUT and digital port connectors. .



Using Analog Input Controls

The table below shows recommended values for analog input to activate the channels. The analog discriminator uses windows 0.5V wide.

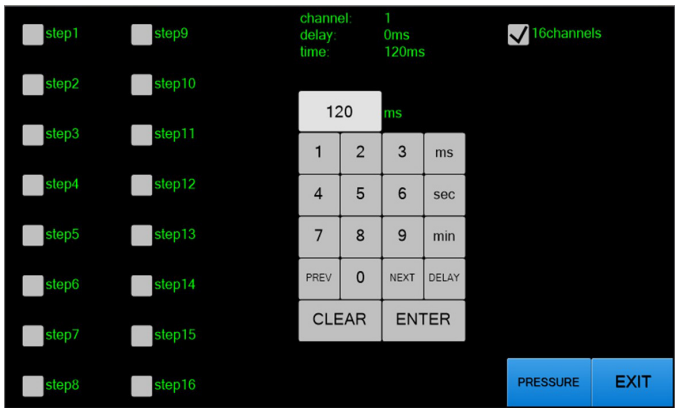
Input Voltage	Channel Activated
0 - 0.5 V	None
0.5 - 1 V	Channel 1
1 - 1.5 V	Channel 2
1.5 - 2 V	Channel 3
2 - 2.5 V	Channel 4
2.5 - 3 V	Channel 5
3 - 3.5 V	Channel 6
3.5 - 4 V	Channel 7
4 - 4.5 V	Channel 8
4.5 - 5 V	Channel 9
5 - 5.5 V	Channel 10
5.5 - 6 V	Channel 11
6 - 6.5 V	Channel 12
6.5 - 7 V	Channel 13
7 - 7.5 V	Channel 14
7.5 - 8 V	Channel 15
8 - 8.5 V	Channel 16

Using TTL Input in CODE Mode

TTL Inputs				Channel Activated
TTL 1	TTL 2	TTL 3	TTL 4	
0	0	0	0	Channel 16
1	0	0	0	Channel 1
0	1	0	0	Channel 2
1	1	0	0	Channel 3
0	0	1	0	Channel 4
1	0	1	0	Channel 5
0	1	1	0	Channel 6
1	1	1	0	Channel 7
0	0	0	1	Channel 8
1	0	0	1	Channel 9
0	1	0	1	Channel 10
1	1	0	1	Channel 11
0	0	1	1	Channel 12
1	0	1	1	Channel 13
0	1	1	1	Channel 14
1	1	1	1	Channel 15

Programming timers and channel sequences

If you touch button SET on the front panel, the display will show SET screen, which will allow you to program timers for individual channels, and delays between channels in sequence.



The touch pad on the right is used to enter time values in ms, sec or min. To switch between time and delay, touch “Delay”/”Time” button. To edit the values, use “Clear” button. Touch button “Enter” to put the new value into the controller memory. In order to mover between different channels values, touch buttons NEXT/PREV.

In order to make all 16 channels available for use, select box “16 channels”. After finishing programming, touch EXIT button.

ONCE PROGRAMMED THE TIMERS SETTINGS ARE STORED in THE CONTROLLER MEMORY and WILL BE AVAILABLE EVERY TIME PC-16 CONTROLLER IS USED. IN ORDER TO CHANGE TIMING or ERASE SETTINGS - THE CONTROLLER NEEDS TO BE RE-PROGRAMMED USING SET SCREEN

Each channel can be programmed for TIME to be active, and DELAY time before it becomes active after the previous STEP in the sequence. If the channel has TIME programmed more than 0ms, it will make STEP (with the same number as the channel number) in the sequence.

Sequence will go from lower STEP number to the higher number STEP, if activated. For sequence to continue to the next STEP, DELAY for this STEP (channel) should be more than 0ms. DELAY of 1ms makes sequence go “without” a delay (with almost no delay). If either TIME or DELAY for a STEP are equal 0ms – sequence will stop before this “not activated” STEP. If DELAY for channel 1 is more than 0ms – sequence will continue as a “loop”, starting again with STEP 1. Up to 16 STEPs can be activated, even if only 8-channels are physically present in the system. If automatic WASH is used between steps while in delay, the maximum number of “steps” will be 32.

To initiate sequence, the controller should be put into AUTOmatic mode, by touching AUTO button on the screen, and then pressing (on the screen or remote) any button (STEP) in the sequence. Removing AUTOmatic mode by pressing the button again, will stop the sequence.

After STEPs in the sequence are activated using SET screen, multiple channels can be added to the STEP, by going through the channels (touching NEXT/PREV buttons) and selecting STEPs where the channel should be active. This can be used to overlap multiple channels at the same time, combine different channels in the same volume/sample, or activate multiple channels to apply solutions in parallel to different samples/volumes.

For more flexibility, the channel can be excluded from the same number STEP by un-selecting the same number STEP on the screen (for the same number channel). This means that although the channel was used to activate the STEP in the sequence, it does not have to be actually OPEN during this step. This can be used to make sequences of channels in random order.

Software control using RS232 port.

After connecting the controller to your computer through provided RS232 null-modem cable (or an optional USB adapter **CFPS-USB**), you can switch valves by sending ASCII codes from your application software. This is used to program automatic perfusion control during your experiment.

RS232 port (COM1, for example) should be set at 115,200 baud, 8 bits, Parity none, 1 Stop bit. The following is the list of commands:

Command	
!Sn	Set channel n ON (where n is 0 for channel 1, F for channel 15)
!Cn	Set channel n OFF
!A0	Set first 8 channels (1 … 8) OFF
!B0	Set second 8 channels (9 … 16) OFF
!A1	Set first 8 channels ON
!B1	Set second 8 channels ON
!SG	Set all channels ON
!CG	Set all channels OFF
!Rn	Read status of channel n, returns 0 if OFF, and 1 if ON
!RG	Read status of all channels, returns 16 bits nnnnnnnnnnnnnnn, the first bit is channel 16, the last is for channel 1

Noise and Grounding

Noise

50/60-Hertz power line noise may be encountered because of:

1. Improper grounding of probes, micro electrodes, bath or instrument chassis.
2. Radiation from transformers of adjacent equipment.
3. Power noise from attached equipment, i.e., stimulators, etc.
4. Antenna effects of cable or wire.
5. Potential difference between various components of electronic set-up (due to the distance electronics are from one another, or different earth grounds).

50/60 Hz noise is not the only electrical signal likely to cause interference problems, some others are:

1. Remote switches, such as in refrigerators or heaters.
2. Voltage pulses emanating from adjacent micro electrodes.
3. Broadcast interference from TV/Radio.

Instrument Grounding

Chassis Ground

(Black): This jack is internally connected to the chassis.

Circuit Ground

(Green): The internal circuitry ground is connected to this jack. A shorting wire connecting the circuit and chassis ground can be used. If ground loops are experienced (objectionable 50/60 Hz) the shorting wire should be removed and the circuit ground jack should be connected to the system ground, which in most cases is a heavy brass bus to which all apparatus grounds are tied. If TTL or analog signals are used, the controller might be already connected to the system ground through the shielding of the cables.

Chassis Grounding

The Chassis of the instrument is not grounded (i.e. floating). The Instrument Chassis can be grounded manually by connecting to the Green back panel grounding jack or to some other system grounds.

Valve Box

The valves are shielded, but the shielding is not connected to any Ground lines. They can be connected to the microscope stage or your system ground inside Faraday cage using a wire connected to one of the crews on the bottom of the box. The cable shielding is connected to the chassis, but not to the valve box. To access the cable shielding directly, find a small wire sticking from under the shell of one of the connectors. Grounding the valve box separately will help to eliminate grounding loops made through shielding.

Custom Modifications

Bioscience Tools can modify and add custom features on request. An example of possible modification is changing TTL control so that high level (+5V) closes and low (0V) opens the valve, or modifying the internal software to work with N.O. valves instead of N.C. ones.

Warranty

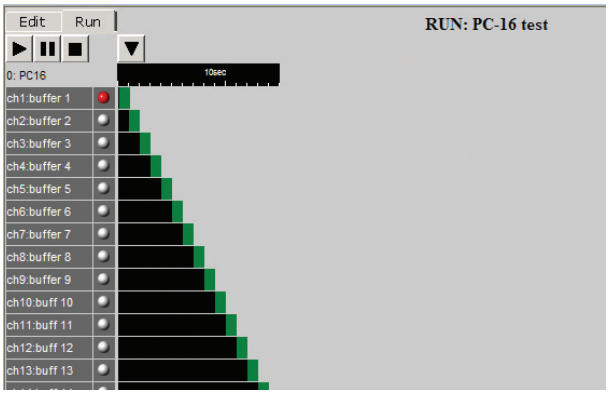
This product is warranted to be free from defects in material and workmanship for the duration of one year. Normal wear, or damage resulting from abuse, accident, alteration, misuse, service by an unauthorized party or shipping damage, are excluded from this warranty and are not covered. Bioscience Tools will repair or replace the defective product covered by this warranty free of charge if it is returned, postage prepaid, to Bioscience Tools, ph: 1-877-853-9755.

Appendix

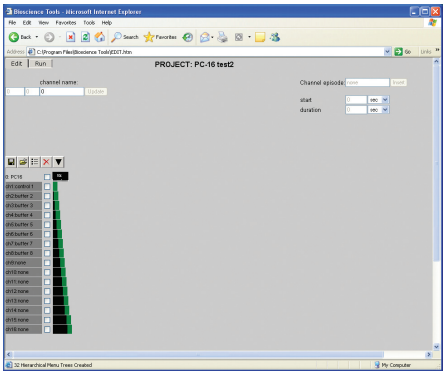
Using Protocol Automation Software

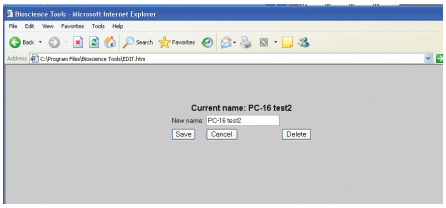
The Protocol Automation software package ships installed on a laptop computer. Connect the computer to the PC-16 controller using provided NULL MODEM cable. Turn the computer ON. Find and double-click BIOSCIENCE TOOLS icon, which is located in the middle of the Desktop. What you will see on the monitor is a sample protocol to activate all 16 channels of PC-16 controller. Click the button PLAY (black triangle) to start the protocol. The controller's channels will be activated for 1sec in sequence. The RED indicators on the controller's front panel and manual buttons on the computer's monitor will be ON for 1sec. After the last channel is OFF, pop-up window will notify you that the protocol is completed.

Manual channel control can be achieved by clicking small round button located next to the name of the channel. This can be used during initial setup of your experiment.

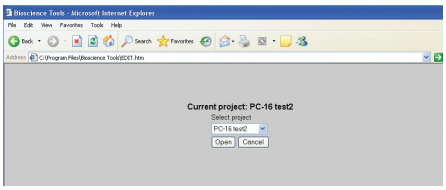


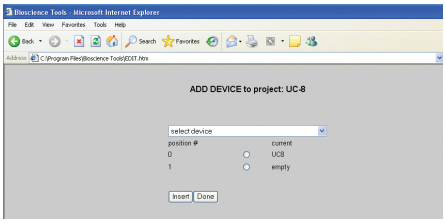
In order to edit or create a new protocol, click the tab EDIT. On the following screen click the button SAVE AS. The following screen will let you to create another protocol under different name. After typing a new protocol name, click button SAVE.



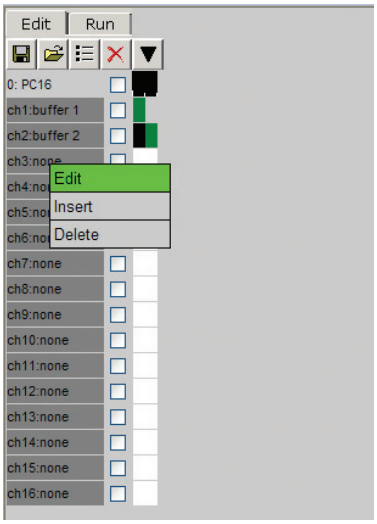


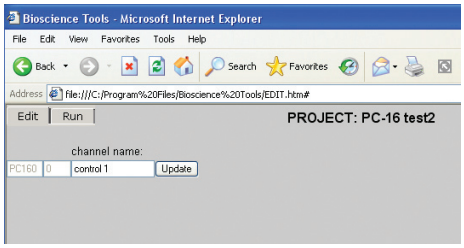
To delete the project, click icon SAVE AS, and click button DELETE on the following screen. To open another project click icon OPEN and select a project from pull-down list. To insert devices into the project, click icon INSERT and select a device from pull-down list.



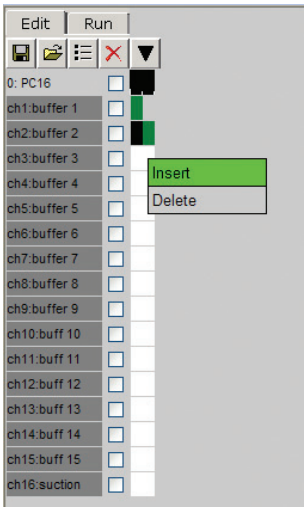


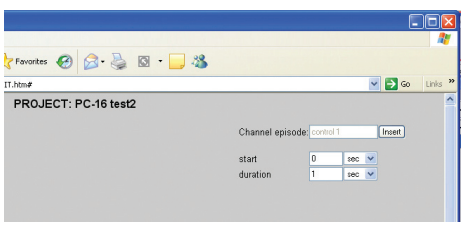
Each channel can be renamed to reflect different settings used during the protocol. In order to rename the channel, click on the channel name, and on the pop-up window select EDIT. In the top-left corner of the screen, type in a new name, and click button UPDATE.



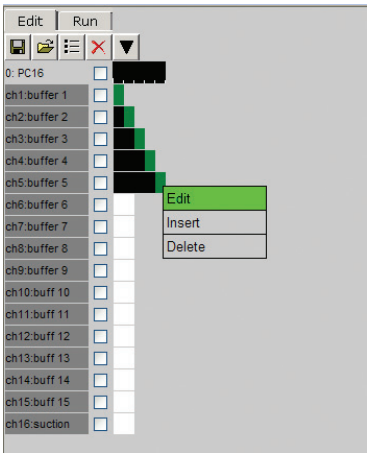


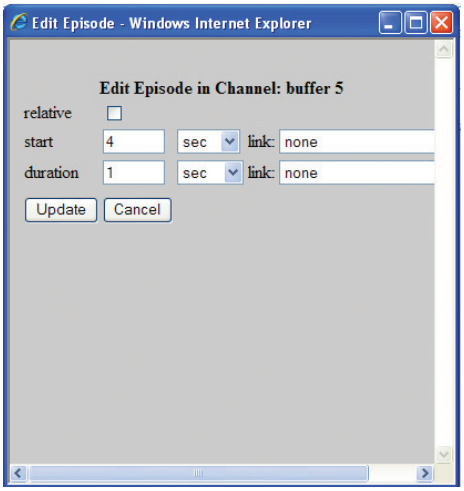
Each channel can have an unlimited number of time episodes, during which it is ON. If the channel does not have active episodes, it will be represented an white square. In order to created an episode, click on the white square and select INSERT on the pop-up widow. In the top-right corner of the screen, type in start time and duration. and click button INSERT. Start time is calculated from the beginning of the protocol. The episode will be represented by a GREEN area.



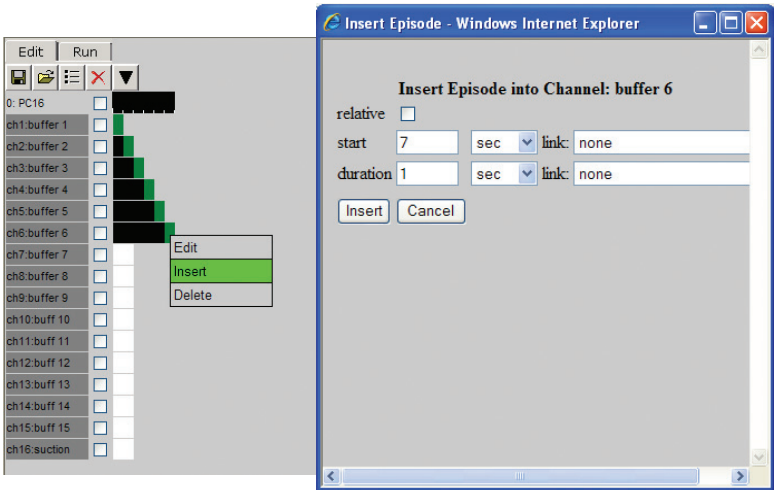


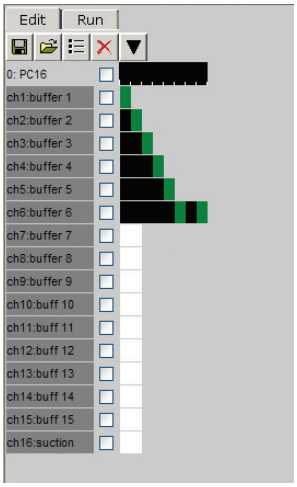
In order to change time settings for an episode, click on the episode and select EDIT on the pop-up. In the top-right corner of the window, type in new time parameters and click button UPDATE.



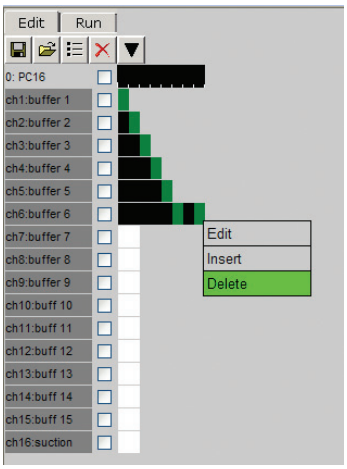


An additional OPEN episode can be added after an existing episode for the same channel. Click on GREEN episode and select INSERT on the pop-up window. In the top-right corner, type in time parameters required and click button INSERT.

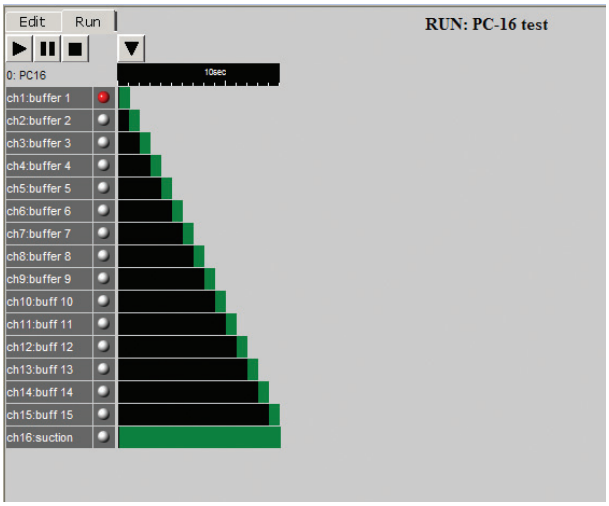




In order to DELETE an OPEN episode, click on the episode and select DELETE. Click button DELETE in the top-right corner to confirm. The episode and corresponding GREEN area will be removed from the screen.



In order to execute the protocol, click tab RUN. On the following screen, click button PLAY to start he protocol.

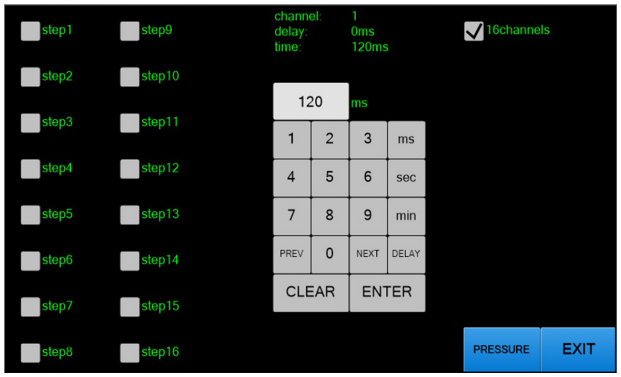


Pressure Controller, built-in PC-16P model

The complete pressure control system comes with a built-in pressure controller, and tubing to connect to custom set-ups. No pressurized source of gas is required to operate the system. During operation, the controller is continuously monitoring the output pressure level to provide consistent and defined flow of solution.

1 Using provided fitting connect 1/16” I.D. tubing to your setup: pressure switch PS-V8P, pressure manifold SH-A, pressure cylinders PC or a small volume delivery system SVDS1/2. Some additional tubing and fitting might be required. Usually some luer-lock fitting or other easy-connect adapters are used to splice different diameter tubing while connecting to 1/8in O.D. BLACK tubing provided, which fits inside the output port with YELLOW rim on the back of the controller. After splicing BLACK tubing to your setup, simply push the tubing inside the port all way, and slightly pull back to clamp. In order to disconnect the tubing, push YELLOW rim inside the connector, and pull the tubing out. Plug the power cable into the wall outlet.

2 Turn the controller ON. Initially, the output is open to the air. Touch SET button and then, PRESSURE button. Set/adjust required pressure using the key-pad and CLEAR button, and touch ENTER key to save the new value. Push button START to start the pressure controller.



3 The output pressure will regulate solution flow rates in your liquid delivery setup.
IMPORTANT: in case of open (not-sealed) systems, it is normal pressure reading being low. This, however, should be avoided since the controller is designed to provide pressure to closed systems in order to regulate flow rates in small volume delivery setups.

Specifications

Output Range

0 to 15 PSI (other ranges available upon request)

Sensors

built-in pressure sensors;

Feedback

from output pressure sensor;

Power Supply

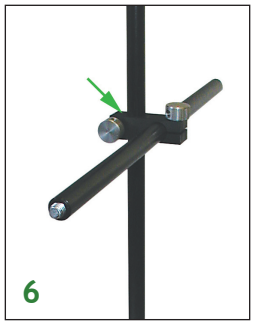
94 to 234 V AC, 50/60 Hz

Syringe Holder, SH-1A

1. SH-1A Syringe Holder comes with a 1x1' vibration isolation stand, magnetic base, three ½" O.D. 1' feet long rods, plastic syringe holder for 50-60ml syringes, 16 syringe adapters for 5-10 ml (x8) and 30-35 ml (x8) syringes, eight syringes, Tygon tubing, stop-cocks, and luer-barb fitting.
2. If using a magnetic base, position the base on top of a magnetic surface.
3. The stand can be directly attached to threaded surfaces using provided M8 screws. If using threaded surface or.
4. Screw 1' rod into magnetic base.
5. Screw another rod to the top of the previous rod. Repeat if extra height is required. Syringe holder can be extended up to three feet high by attaching the rods to each other.
6. If a perfusion system is used, put provided x-blocks on the rod, before attaching the next rod on top.
7. Fix the syringe holder on the top post.

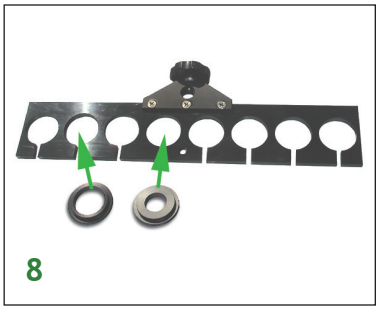

2

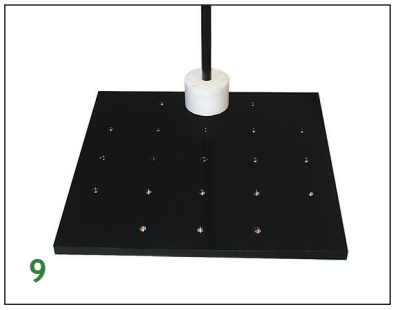

5


6


7

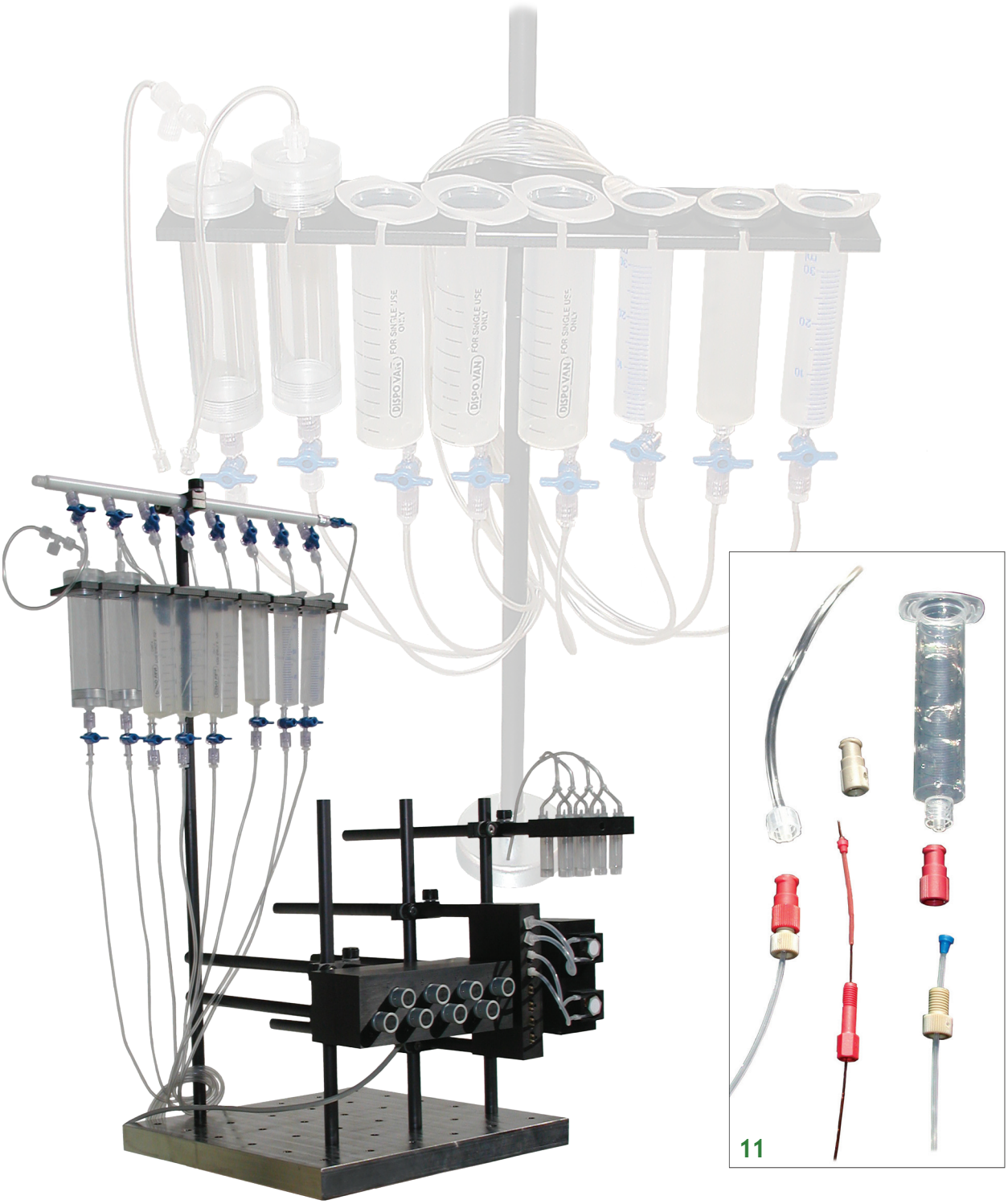
8. Insert syringes into the holder. If smaller volume syringes are used, insert syringes into ring adapters first.
9. The rods can be attached directly to the provided 1x1' stand.


8


9


10

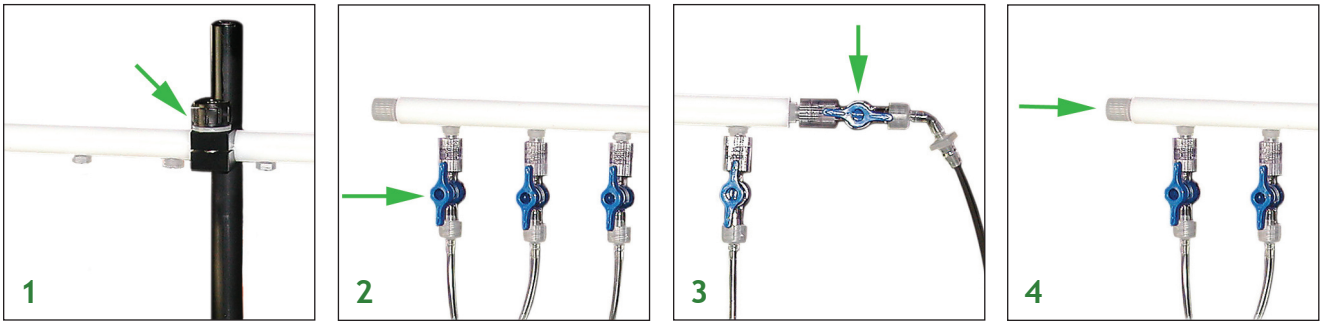
10. Attach stop-cocks to the syringes. Connect TYGON tubing to the barb of luer connector and attach to stop-cock.
11. Connect different tubing using ferrule type and luer adapters: Both Tygon and polyethylene tubing can be connected to the syringes. Soft Tygon tubing fits to provided barbed luer connectors, which lock into the syringes. Polyethylene tubing needs to be attached to ferrule type luer lock adapter first. Micro-bore tubing can be also connected to either syringes directly or to Tygon and polyethylene tubing using provided ferrule type adapters. Note: microbore tubing requires sleeves. Ferrule-based fitting is included with complete perfusion systems, or in optional PS-KIT kit.


DISPOVAN FOR SINGLE USE ONLY
DISPOVAN FOR SINGLE USE ONLY
DISPOVAN FOR SINGLE USE ONLY

11

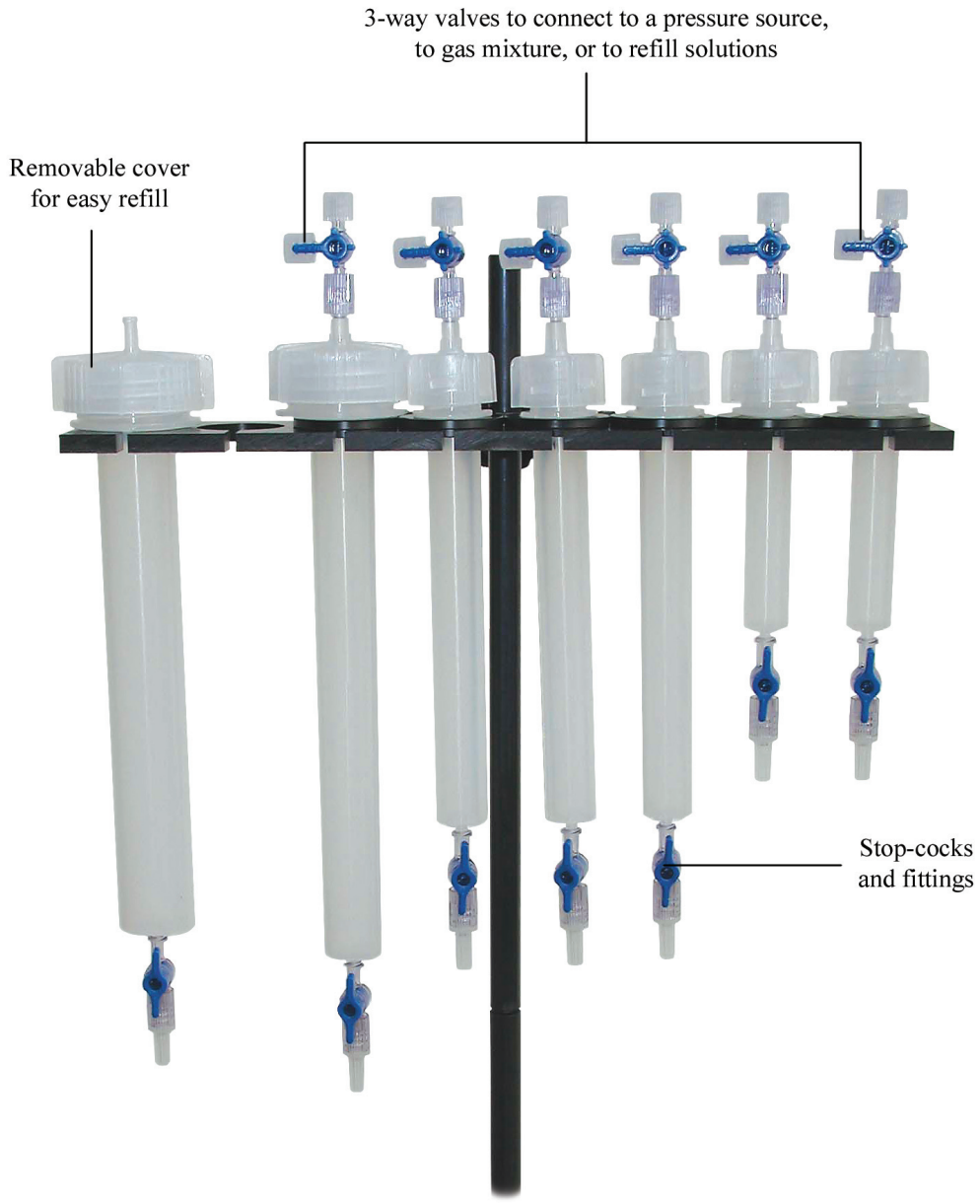
Gas Mixture Adapter- Pressure manifold, SH-A

This adapter is used to pressurize or to deliver gases, CO2/O2 for example, to experimental solutions in syringe barrels or other containers. Continuous bubbling of the experimental solutions ensures gas saturation inside the solutions. The adapter can be also used to pressurize the solutions by connecting to optional pressure cylinders, PC, available in different sizes - volumes.

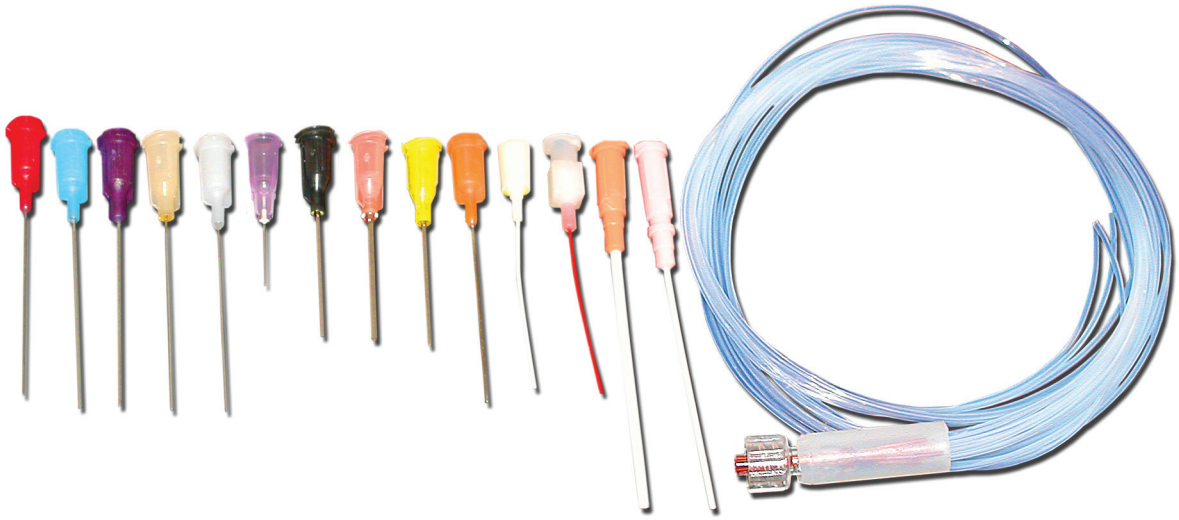


1. Mount the adapter on a 0.5in. post (included with SH-1A syringe holder) using provided X-block.
2. Eight luer connectors positioned along the adapter deliver gases to eight separate solutions through manual valves (stop-cocks) and thin PFTE or polyethylene tubing. The tubing can be replaced with any custom tubing and other means to dissipate gases inside solutions (aquarium stones, for example). If less then 8 solutions are used, the extra outlets can be closed.
3. Use soft tubing with luer-lock to connect to a source of gas mixture through a manual valve attached to the end.
4. Another end can be plugged, or connected to the second adapter (several adapters can be connected in sequence to use the same gas mixture).

Pressurized Cylinders, PC



Luer-Lock Manifold, PM



1. The luer-lock manifold can be used with tips, nozzles or catheters that have standard luer connectors for sample infusion or perfusion. The nozzles can be used to fit inside microbore tubing or chambers. We can provide custom polyimide/Teflon nozzles/catheters of specified length and diameter.

2. The manifold incorporates 2 ft. long connecting tubing with luer connectors for connection to either pressure or gravity driven perfusion system PS.

3. Although adequate solution flow through the manifold will occur even with a regular gravity driven perfusion system, much faster flow rate can be achieved if solutions are pressurized: using small volume delivery SVDS1/2, or flow control systems CFPS.

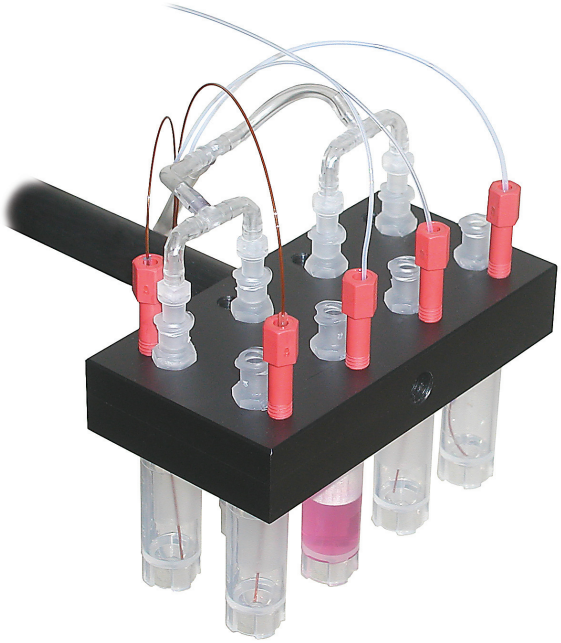
4. The connecting tubing should be attached to a perfusion system using short sleeves made out of soft tubing or fitting from PS-KIT. The tubing should be connected to outlet of valves; the other inlet of the valves should be connected to a source of solution: syringes with solutions held in syringe holder SH-1A for example.

5. If small volume delivery system SVDS1/2 used with a single source of pressure, the manifold should be connected to the valves of PS system first, to brake the lines and to prevent back flow; the other inlet of the valves should be connected to pressurized small volume delivery system SVDS1/2.

6. If used with less different solutions that channels available, some channels can be filled with the same solution, for faster washout of samples for example.

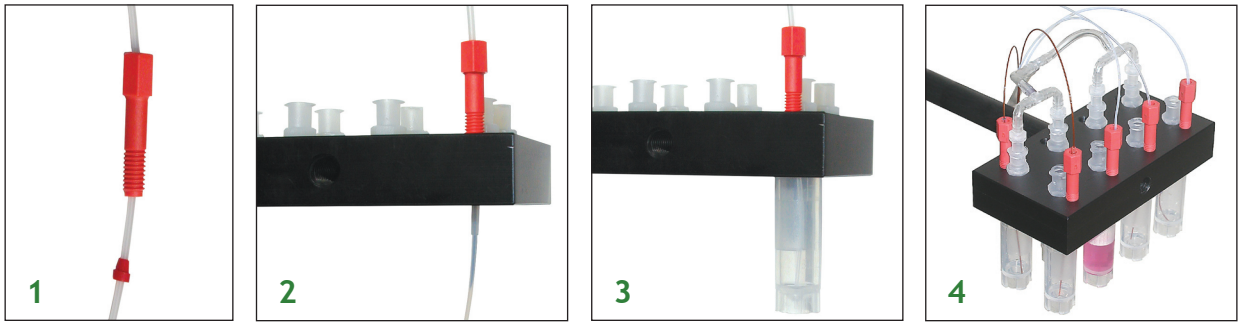
7. Always wash the manifold with DISTILLED water after use.

Small Volume Delivery System, SVDS1/2



SVDS1 system can be used with a pressure source, or solutions can be withdrawn by a negative pressure supplied by CFPS-1U units. The output solution tubing can be connected to valves of a PS solution switch, and then to a MM, PM or ZMM micro-manifolds. The pressure input should be connected to a regulated pressure source using 1/16" I.D. tubing and T/Y-connectors – one pressure input to all eight (or less) pressure input luer ports positioned on the top. The solutions will be switched by turning ON/OFF the appropriate valves by the controller of the perfusion system. The applied pressure will push the solution through the opened line.

The system ships fully assembled. Below are the instructions on connecting the replacement tubing.



1. Measure and cut eight (or fewer) pieces of polyethylene tubing, 1/16" O.D. - fitting sleeves. Put a short piece of the fitting sleeve over delivery tubing (the system is shipped with 2' of Teflon tubing per each channel). Insert the sleeve into the ferrule.

2. Secure the tubing inside the plastic block by tightening the threaded nut (do not tighten completely yet).

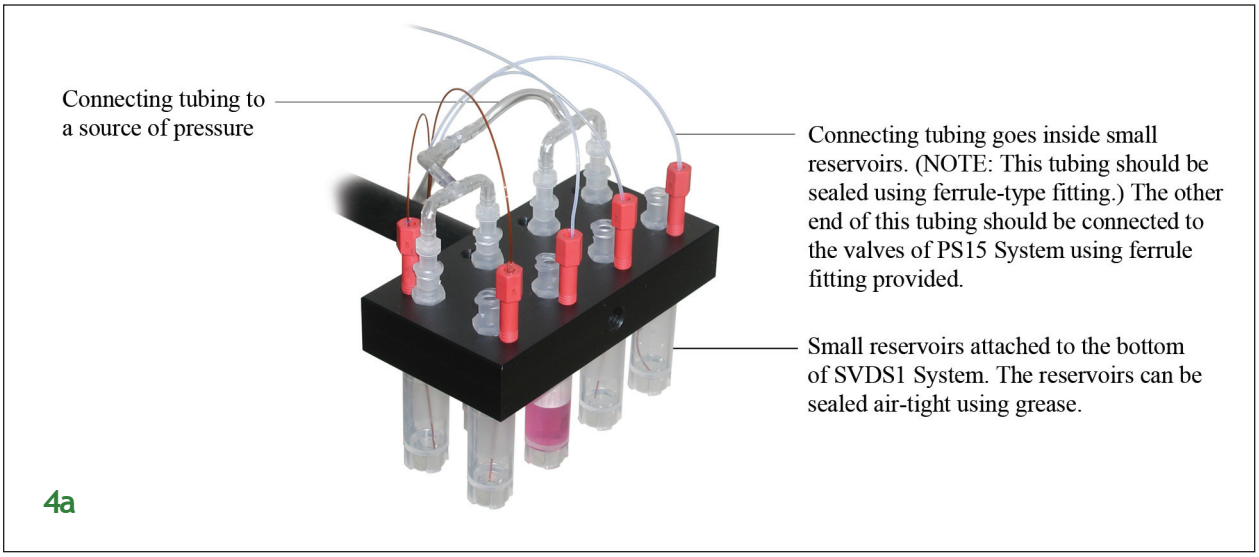
3. Screw in conical plastic tube (included), and pull the delivery tubing so that the end of it still touches the conical bottom. Tighten the threaded ferrule fitting.

ALTHOUGH PROVIDED FITTING WILL ENSURE AIRTIGHT SEAL, THREADED PORTS AND TUBES MIGHT REQUIRE SOME GREASE TO MAKE AIR-TIGHT SEAL INSIDE THREAD.

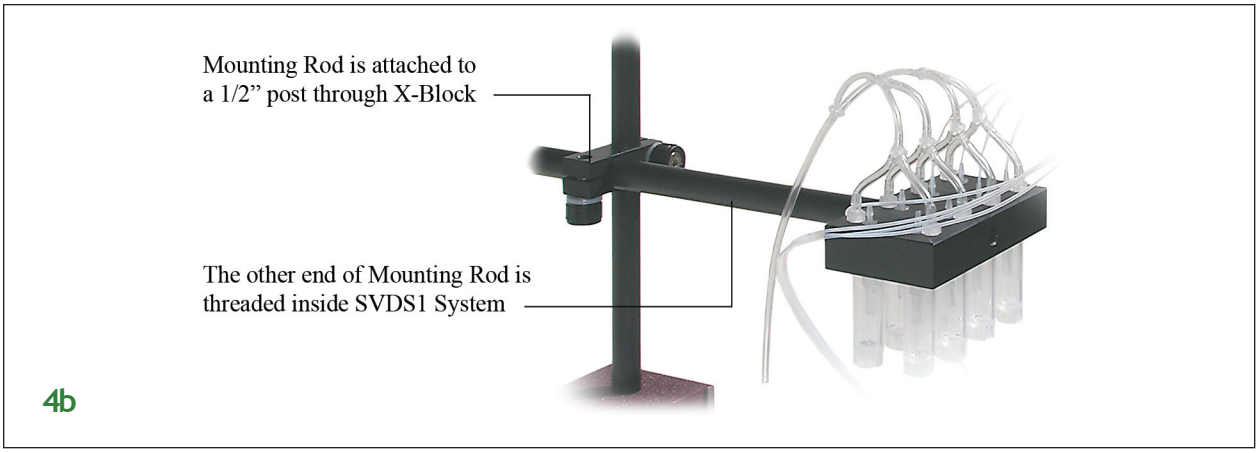
4. The system can be mounted on a custom 6 mm O.D. rod or on 1' long threaded aluminum rod, which can be mounted on a standard 0.5" O.D. stand through X-block (X-block and a threaded rod are included). This allows

positioning the solutions near your samples, to minimize the dead volume.

If valves used to open solution lines, connect Teflon tubing to valve's inlet using sleeves of soft tubing. The valve's outlet should be connected to a micropipette, a micro-manifold or a chamber using another tubing.

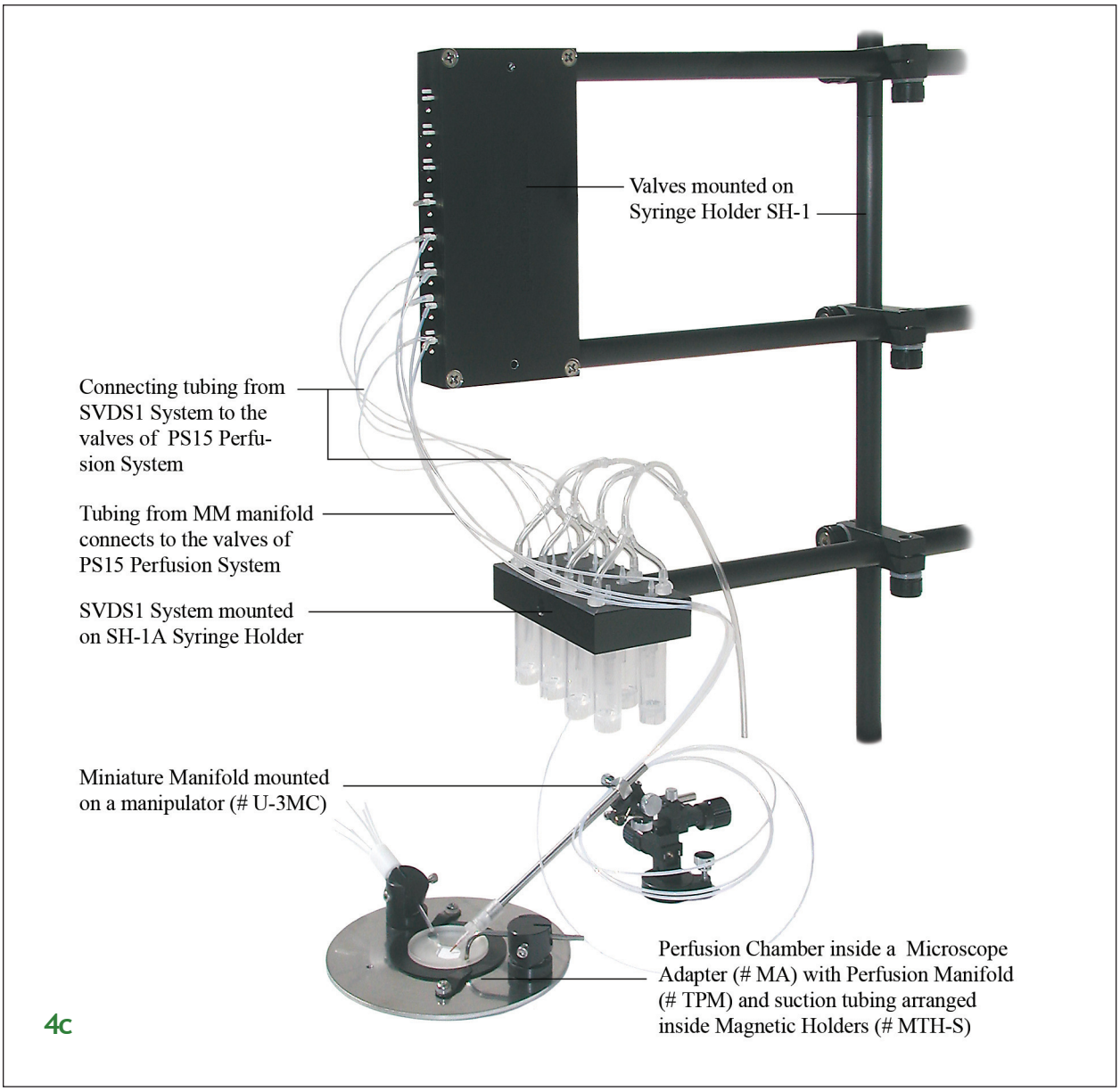


4a



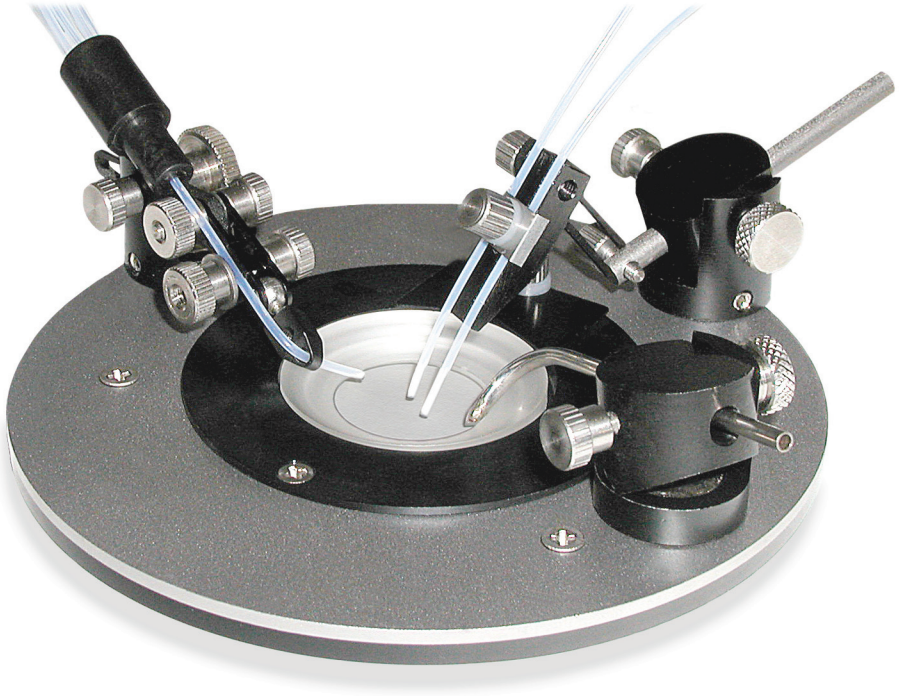
4b

A setup with small volume delivery system SVDS1 and miniature manifold might look similar to this.



4c

Perfusion Manifolds, TPM

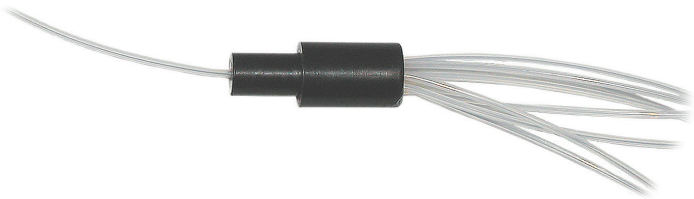


perfusion manifolds are used to switch between multiple solutions during small volume bath perfusion. This item is included with computerized perfusion systems, PS. The manifold comes with short pieces of Teflon tubing, which fits to polyethylene tubing included with perfusion systems. These pieces can be easily replaced with different tubing of your choice. Use ferrule and luer-type fitting from PS-KIT to attach to different tubing, including 1/16” and larger I.D. soft tubing.

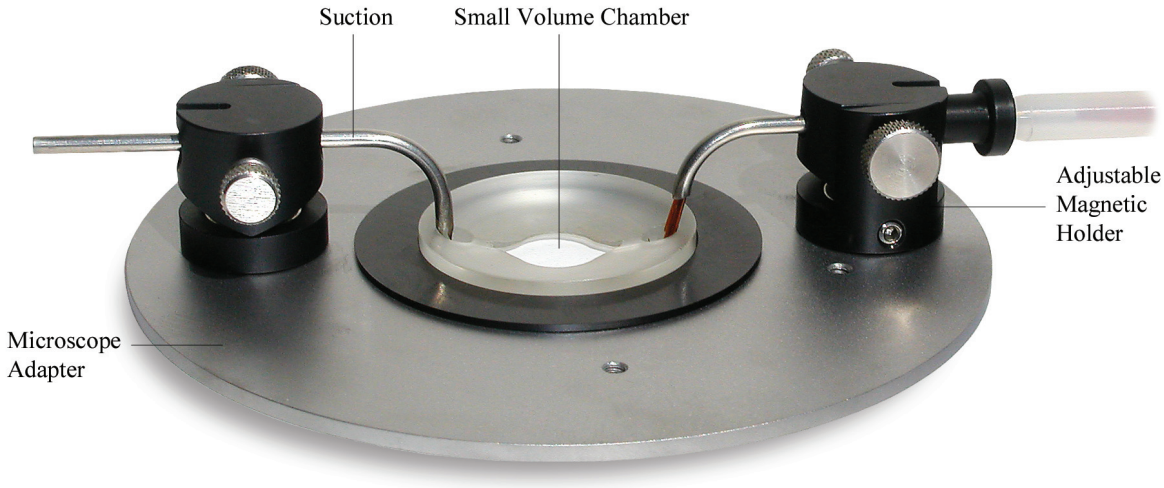
1. Attach the manifold to polyethylene tubing, PPT, by sliding the tubing over short Teflon tubing inserted inside the manifold. This splicing provides leakproof connection to your perfusion system.

2. To prevent contamination, wash the manifold with distilled water after use by filling the perfusion system with water and letting it drain through completely through the manifold. Do not leave water inside the manifold for long time.

Note: The unused channels of the manifold can be filled by provided short pieces of Teflon plugs. Alternatively, the idle channels can be filled with control buffer, for faster wash for example.

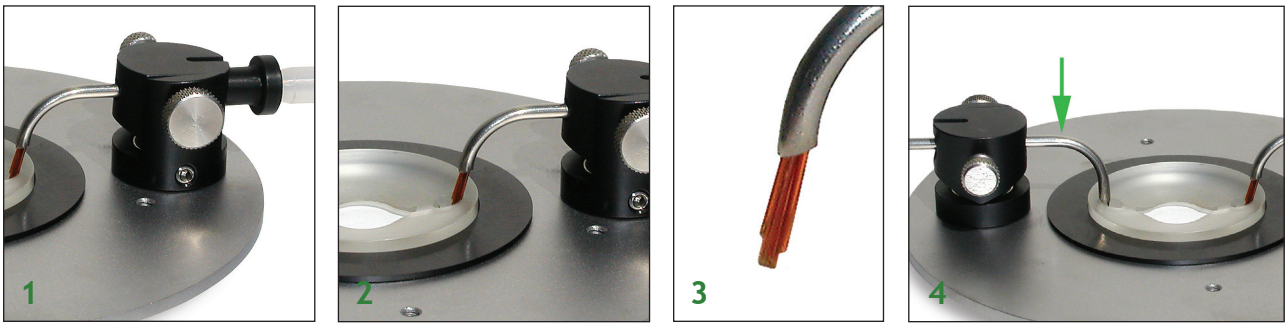


Zero-Dead Volume Manifolds, ZMM



In case when even a slight contamination of different solutions is undesirable, a zero-dead volume manifold can be used instead of regular Teflon manifolds. The zero-dead volume manifolds offer the additional advantage of facilitating perfusion and accelerating solution exchange rate around your samples. This manifold can be also used for solution outflow/suction, by connecting one of the channels to CFPS-1U unit.

ZMM manifolds come with 2 feet tubing with luer connectors, to connect to a computerized perfusion systems, PS. Use fitting from PS-KIT to attach connecting tubing to either 1/16" soft tubing or polyethylene tubing, included with perfusion systems.



1. Position the manifold on a microscope adapter.

2. Place the tip of the manifold inside a chamber. The stainless tubing can be moved inside black holder to obtain desired configuration and to fit inside perfusion chambers.
3. The inside polyimide tubing can be adjusted to provide non-contaminating flow of different solutions by positioning the outputs at different heights. The polyimide tubing can be cut to required length using a surgical grade

sharp blade or a scalpel. Polyimide tubing is washable.

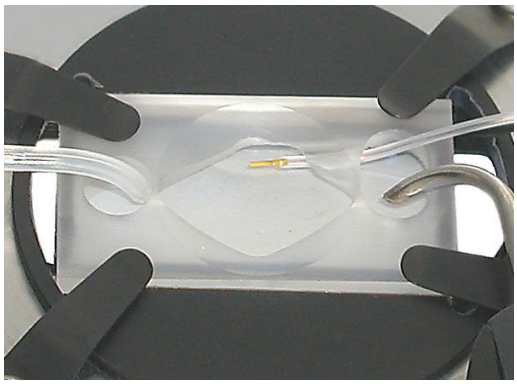
Note: Selected channels of the manifold can be also used to provide suction or outflow of solution from the chamber to keep the volume inside the chamber constant. The height of the suction tubing will determine the level of solution (volume) inside the chamber.

4. Position outflow or suction tubing inside the chamber before using the perfusion system.

Note: The polyimide 360/250 micron I.D. tubing inside the manifolds will provide adequate solution flow with regular gravity driven perfusion system PS. If higher solution flow rate through the manifold are required, the solutions can be pressurized or elevated. SVDS1/2 systems, for example, requires external pressure application to drive the solutions from the reservoirs. Controlled flow systems CFPS can also provide enough pressure to drive the solutions through the manifold.

Note: Always wash the manifold with DISTILLED water after use.

Laminar profile perfusion chambers, PLD22x40



This is a chamber that provides laminar solution flow for your sample. Thin profile and wide working compartment will fit under water immersion objective. Thin glass bottom allows you to use oil-immersion objectives. Combined with miniature holders, forms a perfusion system. Requires a microscope adapter; specify microscope model when ordering. Separate inflow and outflow compartments prevent bubbles from entering inside working compartment. Consider adding perfusion or controlled flow system and outflow unit for suction to remove solution during perfusion. Requires PLD-A adapter.

Specifications:

Outside dimensions: 22x40mm

Height: 3mm

Working volume: 14x20mm, approx. 280 microl

Separate inflow compartment. Keeps temperature up to flow rates of 2ml/min

Laminar profile perfusion chamber, PLD22x40

Silicone chamber with laminar cutout to provide smooth perfusion. Attached to a coverslip using a removable adhesive layer. Thin 3mm profile allows you to use water immersion optics and access samples for recordings, injection and local substance application. Separate inflow and outflow compartments prevent bubbles from entering the working volume during perfusion. Incorporated channel for reference electrodes or temperature probes. Requires a

mounting adapter PLD-A and a microscope adapter. Consider adding an outflow unit for suction to remove solution during perfusion. The heated version of the chamber is also available.

Adapter for laminar perfusion chamber, PLD-A

This adapter allows you to use laminar flow chamber PLD22x40 with any microscope adapter that have a 50mm cut-out. The microscope adapters can be used to mount miniature holders for different accessories. Comes with for sets of clamps and mounting thumb screws to fix the chamber.

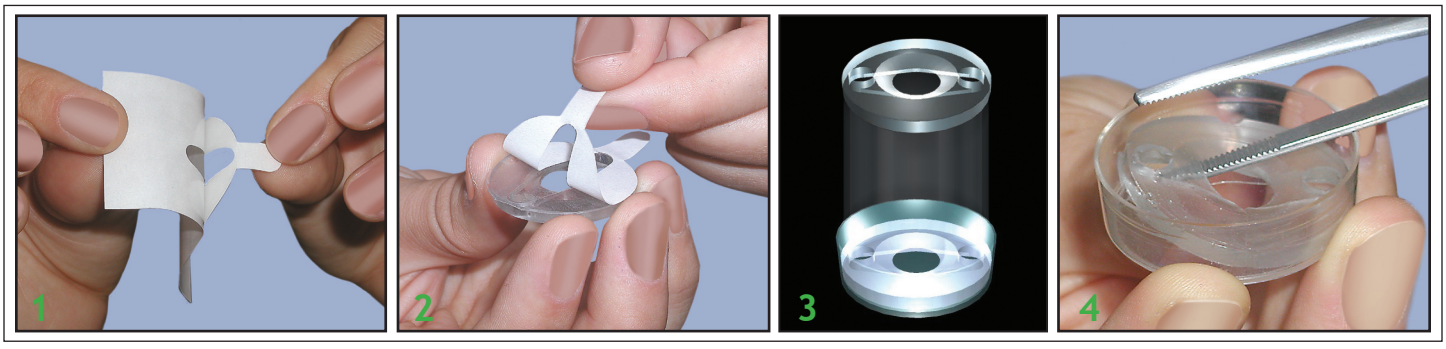
Specifications:

Petri dish inserts PDI

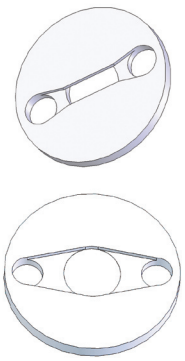
Catalog #	Features:
PDI	Silicone insert with laminar profile for solution flow. Separate inflow and outflow compartments to prevent bubbles from entering the working volume. Includes pack of 50 self-adhesive gaskets. Fits 35 and 50mm dishes.
volume	100 µl
height	3 mm
clearance	11 mm, conical cutout in the center to provide easy access to the sample

Petri Dish Insert is designed to facilitate solution exchange inside standard culture dishes. This flexible silicone chamber fits most Petri dishes. The insert ships along with self-adhesive gaskets, which form airtight and leak-proof contact with the bottom of the dish, even if the dish is filled with media and if you use plastic dishes with uneven surface on the bottom. This effectively eliminates substance trap and contamination during media exchange. The standard configuration has a laminar cutout to provide fast solution exchange inside the dish without bubbles entering the working volume.

We provide Custom Designed inserts with solution flow profiles specific to your application requests, including closed configurations to form defined sheer stress to the samples on slides or coverslips.



Instructions for using Petri Dish Inserts - PDI, LPPCP1, TC-PCP



1. Pull the tab to release the adhesive layer from the protective liner. 2. Align the adhesive along the flow cutout on the bottom of the insert, and remove the remaining protective liner by carefully pulling the tabs. 3. Put the insert inside the dish. **If the dish is filled with liquid media, press the insert firmly down for at least 10sec.** Position perfusion accessories inside inflow and outflow compartments to form solution flow. 4. After the experiment, use forceps or any other appropriate tool to remove the insert from the dish by pushing the tool under the insert and gently pulling it up.

Examples of PDI use inside a microscope heating stage in combination with perfusion

Instructions for LPPCP1 systems

1. First, release the gasket from the protective liner, by pulling one of the tabs. Second, align the gasket with opening on the bottom of the silicone insert. Attach the gasket to the insert by pressing along the surface of the gasket. Third, remove the remaining protective liner by pulling one of the tabs, while making sure the adhesive remains attached to the insert. Separating a little bit of the adhesive from the protective liner with tweezers might help this procedure. Put the insert inside a dish, make sure the adhesive faces bottom of the dish.

If patch clamping, remove any grease from the surface of the working volume with a small piece of filter paper. The reference electrode or Agar bridge should be positioned inside inflow compartment.

2. Connect outflow unit CFPS-1U66 to the suction tubing. Position the metal suction tubing inside one of the openings in the chamber (without reference electrodes or other probes). The suction tubing should not touch the walls of the insert or any other surfaces (electrodes, for example). Otherwise you can suck all solution through surface tension.

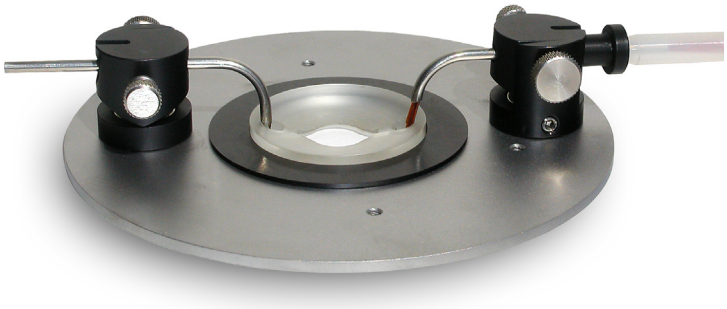
3. Insert the inflow manifold into the magnetic holder. Position inflow tubing into another opening in the holder's ball-joint. Turn ON perfusion and adjust the height of suction tubing if necessary.

4. In order to remove the insert, use tweezers to gently pull one side of the insert up.

5. The stainless microscope adapter will also fit 50mm glass bottom sterile dishes. PDI insert will fit inside 50mm dishes with 40mm optical clearance.

6. The provided clamps can be used to fix the dish firmly in place, which might be useful if oil immersion objectives are used with inverted microscopes, for example.

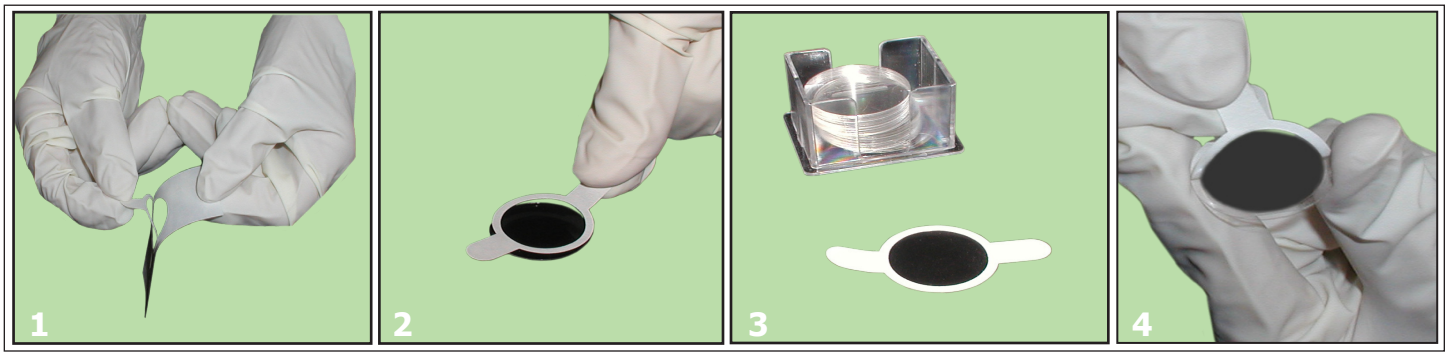
Low-profile Laminar Perfusion Chamber - PCCS2

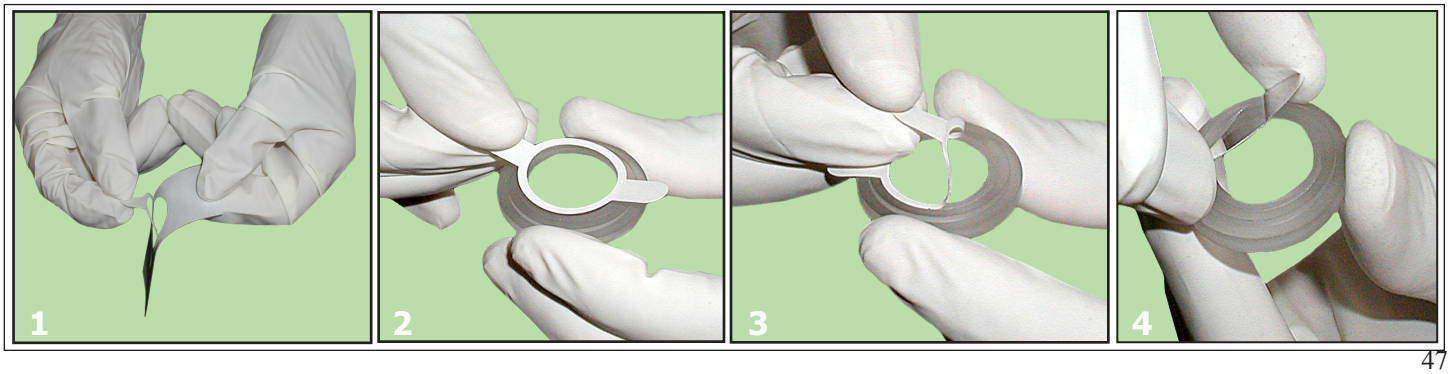


Low-profile chamber inside microscope adapter MA. Miniature magnetic holder with suction tubing MTH-S and zero-dead volume manifold ZMM are positioned around the chamber to form a perfusion system. PCCS2 chamber has separate compartments for inflow and outflow. This prevents bubbles from entering the working volume and provides smooth perfusion. Laminar profile facilitates solution exchange.

1. Remove protective liner from the bottom surface to expose the adhesive.
2. Apply the adhesive side down onto the surface of a coverslip. Press gently to seal. This can be done even if the coverslip contains media with your sample.
3. Add media and/or sample onto the coverslip if some incubation is necessary. Otherwise proceed to step 4.
4. Remove the remaining protective liner, and put the plastic holder on top of the coverslip. Press gently to seal.

Catalog #	Features:
PCCS2	Low-profile plastic perfusion chamber-holder for 25mm coverslips. Separate compartments for inflow and outflow to prevent bubbles to enter the working volume.
UTIC-21	Replacement adhesive layers, x100
PCCS2-PDI	Replacement adhesive layers for PCCS2 chamber, x100





Alternatively, if the sample does not have to be cultured on the coverslips, for example, you can place adhesive layer onto the plastic holder first, and then attach the coverslip to the holder:

1. Remove protective liner from the bottom surface to expose the adhesive.
2. Apply the adhesive side down to the bottom of the holder. Press gently to seal.
3. Remove the remaining protective liner.
4. Position a coverslip on the holder. Press gently to seal.
5. Position your sample inside the chamber.
6. Arrange inflow tubing, suction tubing and reference electrodes or probes around the sample using magnetic holders.
7. Adjust the angle of suction tubing using screws in the holder.

The chamber can be used with both stainless magnetic MA and universal IMA microscope adapters. The microscope adapters provide adjustable clamps, which can be used to fix the chamber firmly in place. This is especially useful if oil immersion objectives of inverted microscopes are used.

Example of using Low-profile chamber together with miniature holder MH-2. This multi-holder is capable of positioning several tubing around the chamber to form a perfusion system.